Synthesis of Lignin-Carbohydrate Model Compounds and Neolignans

by

Kaichang Li

Dissertation submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
IN
WOOD SCIENCE AND FOREST PRODUCTS

APPROVED:

Richard F. Helm, chair

Charles Frazier

Richard D. Gandour

Wolfgang G. Glasser

Robert H. White

September 20, 1996
Blacksburg, Virginia

Keywords: Synthesis, Lignin-carbohydrate, model compounds, uronic acid, benzodioxane, neolignans

Synthesis of Lignin-Carbohydrate Model Compounds and Neolignans

by

Kaichang Li

Committee Chair: Richard F. Helm

Department: Wood Science and Forest Products

(ABSTRACT)

Woody plants are the most abundant renewable resources on the earth. From the paper we consume to the house we live in, our daily lives rely heavily on woody plants. Over the past decades, enormous efforts have been expended to improve the utilization of fiber and wood. For example, much research has been conducted to develop environmentally benign, and economically feasible techniques for pulp and papermaking. The economical conversion of wood to useful sugars and alcohol has also been the subject of intensive research. Investigations aimed at the genetic manipulation of wood growth to better meet our needs are also underway. Nonetheless, harsh pulping and bleaching conditions are still required in the pulp and paper industry, and the bioconversion of polysaccharides in biomass to alcohol is still too expensive. An argument could be put forth that a major reason for this is the lack of basic knowledge concerning the structural and biochemical characteristics of the plant cell wall.

The three major polymeric components of plant cell walls, cellulose, hemicellulose and lignin, are intimately associated with one another. Cellulose is associated with hemicellulose via non-covalent linkages, whereas lignin is theorized to be associated with cellulose and hemicellulose via both covalent and non-covalent linkages. The nature of associations between wood polymers is still poorly understood. However, it is these intimate associations that make delignification difficult, and make the bioconversion of polysaccharides to alcohol inefficient. It is also believed that the linkages between lignin and polysaccharides are responsible for the reduced digestibility of grasses by ruminants.

Besides cellulose, hemicellulose and lignin, there are many secondary metabolites such as lignans, neolignans, tannins and terpenoids. The structures of lignans and neolignans are analogous to the interunits of lignin. Lignin is considered an optically inactive polymer, whereas lignans and neolignans are optically active small molecules. Although it has been proposed that the biosynthesis of lignin, lignans and neolignans are via the same oxidative coupling mechanism, it is still unclear that how the plant cell wall differentiates the formation of lignans, neolignans and lignin. How and why plant cell wall generates so many lignans and neolignans having broad structural variation is also unknown. As a matter of fact, it is still uncertain which enzymes are actually involved in the biosynthesis of lignin. A better understanding of biosynthetic pathways of lignin, lignans and neolignans is a prerequisite for the genetic manipulation of plant growth.

Investigations described in this dissertation were an effort to better understand the fundamental aspects of covalent linkages between lignin and hemicellulose in wood. Enantiomeric synthesis of neolignans provides a tool for investigating the optically active nature of neolignans, and may be helpful to study the biosynthetic pathways of neolignans.

Chapter 1 describes chemical structures of wood components and the biosynthesis of lignin, lignans and neolignans. The mechanisms of lignin-carbohydrate bond formation are also discussed, and a concise review of lignin-carbohydrate linkages proposed in the literature concludes Chapter 1. Chapter 2 presents the methods used in investigating covalent linkages in wood, which include methods of isolating lignin-carbohydrate complexes, chemical cleavage methods, DDQ oxidation and model compound/NMR methods. The synthesis of plant cell wall model compounds and neolignans are reviewed in Chapter 3.

The experimental work performed for the completion of this thesis is described in

Chapters 4-8. A method which provides β-*O*-4 lignin model dimers with complete *threo* stereospecificity is described in Chapter 4. This method is complementary to the current method for the preparation of *erythro* lignin model dimers. Chapter 5 presents a practical synthesis of methyl 4-*O*-methyl α-D-glucopyranosiduronic acid. Methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid was prepared from methyl α-D-glucopyranoside in 4 steps (74% overall yield). Previous preparations of this compound were much lengthier, and had very low overall yields. Chapter 6 deals with the synthesis and rearrangement reactions of ester-linked lignin-carbohydrate model compounds. A series of ester-linked lignin-carbohydrate model compounds were synthesized, and migration of the uronosyl group between the primary (γ) and benzyl (α) position of lignin side chain is discussed. Several approaches to synthetic neolignans are described in Chapter 7. Chapter 8 presents a novel approach for the preparation of chiral aryl alkyl ethers. The successful application of this novel approach to synthesis of several optically active 8-*O*-4 neolignans and a 1,4-benzodioxane neolignan is described, as is the introduction of an alkyl aryl ether bond in carbohydrate molecules.

Some of the material of this dissertation has been reported in the following papers:

1. Li, K. and Helm, R. F. Approaches to Synthetic Neolignans. *J. Chem. Soc. Perkin Trans 1*. Accepted.
6. Li, K. and Helm, R. F. Use of Carbohydrates as Building Blocks to Synthesize Neolignans. 211th ACS National ACS Meeting, New Orleans, March 24-28, **1996**. CELL-079.
2. Li, K. and Helm, R. F. A Practical Synthesis of Methyl 4-*O*-Methyl-α-D-Glucopyranosiduronic Acid. *Carbohydr. Res.* 273(**1995**), 249-253.

3. Li, K. and Helm, R. F. Synthesis and Rearrangement Reactions of Ester-Linked Lignin-Carbohydrate Model Compounds. *J. Agric. Food Chem.* 48(**1995**), 2098-2103.

4. Helm, R. F. and Li, K. Complete *threo* Stereospecificity for the Preparation of β-*O*-4 Lignin Model Dimers. *Holzforschung.* 49(**1995**), 533-536.

5. Helm, R. F. and Li, K. Synthesis and Rearrangement Reactions of Lignin-uronic Acid Model Compounds Related to Hardwood Cell Wall Structure. The 8th International Symposium on Wood and Pulping Chemistry. Helsinki, Finland, June **1995**, vol. 1, pp107-114.

7. Li, K. and Helm, R. F. Approaches to Synthetic Neolignans, 34th National Organic Symposium, Williamsburg, VA. June 11-15, **1995**. Poster 281.

This dissertation is dedicated to my great father, who passed away before he could see his son obtain a PhD.

ACKNOWLEDGEMENTS

First of all, I would like to express my deepest thanks to Prof. Richard F. Helm for his guidance and help. He played a very important role in my understanding of the US. Through his patient help, I gained much knowledge in various aspects of science and western culture. He was the perfect advisor. He not only taught me computer skills, writing skills and efficient working at the lab, but also shared with me his enthusiastic approach to science. His encouragement, friendship and support made my efforts both rewarding and enjoyable.

My gratitude also go to Prof. Wolfgang G. Glasser for giving me the opportunity to come to the USA. Although I could not fulfill my initial wish to work with him in the utilization of lignin, his help and guidance are greatly appreciated. I will always regard him as one of the most respectable professors.

Thanks go to Prof. Charles Frazier for his advice, help and friendship. I found that he well understood what I wanted and where I were heading in my career although we didn't talk too much. I know that he is one that I can trust. I am proud of our friendship and greatly appreciate his help on my committee.

I would also like to thank Prof. Robert H. White (Department of Biochemistry) for being on my committee and for his kindness. My deepest thanks also go Prof. Richard D. Gandour (Department of Chemistry) for his consenting to be on my committee. I would like to thank Prof. Tomas Hudlicky (now at the University of Florida) for his lecture in organic synthesis and his serving on my committee in the early stage of my PhD study. I am always afraid of him, but I learned much in organic synthesis under his huge pressure.

My deepest thanks also go to Mr. Tom Glass for his help in my NMR experiments. Without Tom's help, I would have had to spend a much longer time in finishing my research work.

Thanks also go to Mr. Ralph Amateis (Department of Forestry) for his help in my spiritual growth. He showed me God's power of love and the brotherhood. He helped me establish a good relationship with God.

I would like to express my deepest thanks to my mother for her love. She took pains to raise my bothers and me during the darkest period of Chinese history. She taught me "never give up your hope" no matter how terrible the situation. My special thanks go to my cousin, Dr. Tsaiting Lee (Taiwan) for his generous financial support. He treated me like his own bother. My deepest thanks also go to my Uncle, Yunwa Lee (Hawaii), and my cousin, Dr. Hsing-chung Lee (National Health Institute) for giving me the crucial help to come to the USA, for their care and encouragement during my stay in the USA. I would also like to thank my cousin, Mr. King-shing Li (New Zealand), for his hospitable reception during my stay in Hong Kong. He is my model of pursuing success. I would like to thank my mother-in-law for taking good care of my daughter during my stay in the USA.

I would also like to thank Mr. Ackah Toffey for his friendship. He reminded me that I was not the only one trying to obtain a PhD when I came to the lab on Saturday. We used to work in the same building, and talked often about our American dream. With a friend like Mr. Ackah Toffey, I felt less lonely in the USA.

Finally, I am also grateful to Dr. Geza Ifju (Department Head) and Debbie Garnard

(Secretary) for their kindness and help. My thanks also go Dr. Gamini Samanarayake for his kind help in the early stages of my research. I would also like to thank Mrs. Jody Jervis for her kind help at the lab. I greatly appreciate the great time I have spent with Mrs. Jody Jervis, Dr. Thilini Ranatunga and Mrs. Lourdes M. Orejuela (Ecuador) at the lab. My thanks also go to Dr. Rajesh K. Jain, Mr. Jason Todd, Robert Schmidt, Jianwen Ni and Miss Ulli Becker for their help during my stay in Blacksburg.

CONTENTS

Chapter 8: The preparation of chiral aryl alkyl ethers

Chapter 1

Chemistry and Biochemistry of Wood Components

1.1 Chemical structures of wood components

1.1.1 Cellulose

Cellulose is the most abundant renewable natural resource in the world. About 40%-50% of wood consists of cellulose, which is a linear polymer linked by a (1→4) β-D-glucopyranosidic bond. The linear cellulose molecules easily form inter- and intramolecular hydrogen bonds. Gardener and Blackwell (1974) found that a cellulose chain has a twofold screw axis of symmetry, and is stabilized and stiffened by inter- and intramolecular hydrogen bonds. The easy formation of inter- and intramolecular hydrogen bonds provides cellulose with crystal-like properties. Cellulose molecules in wood exist in both crystalline and polymorphous forms. It has been found that cellulose exists in several crystalline forms which provide different X-ray diffraction patterns and spectra (Blackwell 1982, Atalla and van der Hart 1984). Although it is well understood that hydrogen bonds play important roles in the arrangement of cellulose chains, the exact crystalline forms of cellulose have still not been defined. Crystalline cellulose is highly resistant to hydrolysis and biodegradation, whereas amorphous cellulose is hydrolyzed and biodegraded much faster. Although cellulose is composed solely of glucose, the repeat unit of the cellulose chain is a cellobiose unit (Tönnesen and Ellefsen 1971; Blackwell 1982). While cellulose is known to hydrogen bond to itself and other celluloses, it is also possible that it can hydrogen bond with the other polysaccharides present in the cell wall namely the hemicelluloses.

1.1.2 Hemicelluloses

1.1.2.1 Introduction

The hemicelluloses are both linear and branched heteropolymers composed of D-glucose, D-mannose, D-xylose, L-arabinose, D-galactose and D-glucuronic acid. Composition and linkages among these sugars vary from one plant species to another, and also vary with different parts of a single plant (roots, stems, leaves, wood and bark). Therefore, it is impossible to provide a precise structure of hemicelluloses. However studies over the past several decades have shown that wood hemicelluloses have major structural features, and these have been highlighted in comprehensive reviews (Timell 1964, 1965, 1967; Fengel and Wegener 1989). This review will focus on some specific structural features of hemicelluloses that pertain to hemicellulose-lignin interactions.

1.1.2.2 Softwood Hemicelluloses

Softwood hemicelluloses consist of galactoglucomannans, arabino-4-*O*-methylglucuronoxylan, arabinogalactan and a minor amount of other polysaccharides. Galactoglucomannan is one of the major components in softwood hemicelluloses (about 20%). A general structural formula of softwood galactoglucomannans is presented in Figure 1.

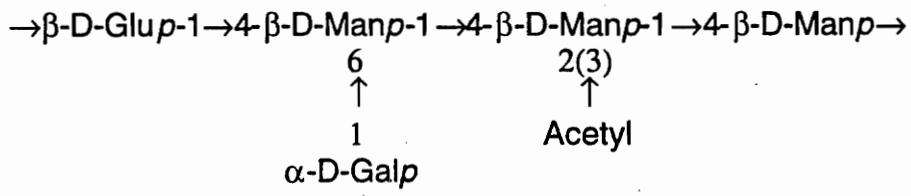


Figure 1. Representative structure of galactoglucomannan (after Timell 1967).

Galactoglucomannan has a heterogeneous backbone that is composed of (1→4)-linked β-

D-glucopyranose and β-D-mannopyranose residues (Jones and Painter 1957, 1959). The ratio of D-glucose and D-mannose in galactoglucomannan is about 1:3 (Timell 1967). Galactoglucomannans in softwoods differ mainly in their galactose composition. Softwood galactoglucomannans bear occasional galactose side chains linked α-(1→6) to the mannose units on the backbone. The fact that galactose exists as a non-reduced end group has been used to characterize covalent linkages between lignin and carbohydrates (Eriksson and Lindgren 1977; Eriksson and Goring 1980). The α-(1→6) linkage of galactose is very sensitive to acid and alkali, and may be cleaved during alkaline extraction (Timell 1965). All galactoglucomannans are probably acetylated in their native state (Koshijima 1960), and the acetyl groups are attached to the mannose residues (Meier 1961). This information can be used in characterizing the linkages of lignin to glucose and mannose units in lignin-carbohydrate complexes (LCC) isolated from softwood by chemical methods such as Smith degradation (Yaku *et al.* 1981).

Besides galactoglucomannans, softwood hemicelluloses also contain arabino-4-*O*-methylglucuronoxylan. Arabino-4-*O*-methylglucuronoxylan is difficult to isolate in a pure state and in good yield. A general structural formula of arabino-4-*O*-methylglucuronoxylan is presented in Figure 2.

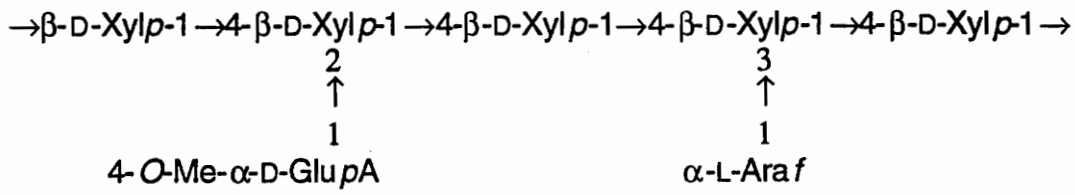


Figure 2. Representative structures of arabino-4-*O*-methylglucuronoxylan (after Timell 1967).

Arabinoglucuronoxylan consists of (1→4)-linked β-D-xylopyranose residues. A terminal 4-*O*-methyl-α-D-glucuronic acid is attached to C-2 position of xylose unit in the backbone.

This type of linkage is similar to that present in hardwood hemicelluloses. Unlike hardwood hemicelluloses, softwood arabino-4-*O*-methylglucuronoxylan also contains α-L-arabinofuranose residues linked to C-3 position of the xylose (Marchessault *et al.* 1963). Arabino-4-*O*-methylglucuronoxylan contains a 4-*O*-methylglucuronic acid unit every five to six xylose residues and an arabinose unit every eight to nine xylose units. Both the arabinofuranosyl and glucuronopyranosyl units form non-reduced ends. As the α-L-arabinofuranosidic linkage is much more sensitive to acid hydrolysis than the other glycosidic linkages and can be selectively cleaved under mild acid hydrolysis. In contrast to hardwood xylans, acetyl groups are not present in arabino-4-*O*-methylglucuronoxylan (Meier 1961). The interference of acetyl groups can probably be eliminated in the investigation of ester linkages between lignin and 4-*O*-methyl glucuronic acid in the acidic fraction of LCCs isolated from softwoods.

All larches contain large amounts of water-soluble arabinogalactan. The arabinogalactan is a very complicated and highly branched polymer. A simplified structural formula is presented in Figure 3.

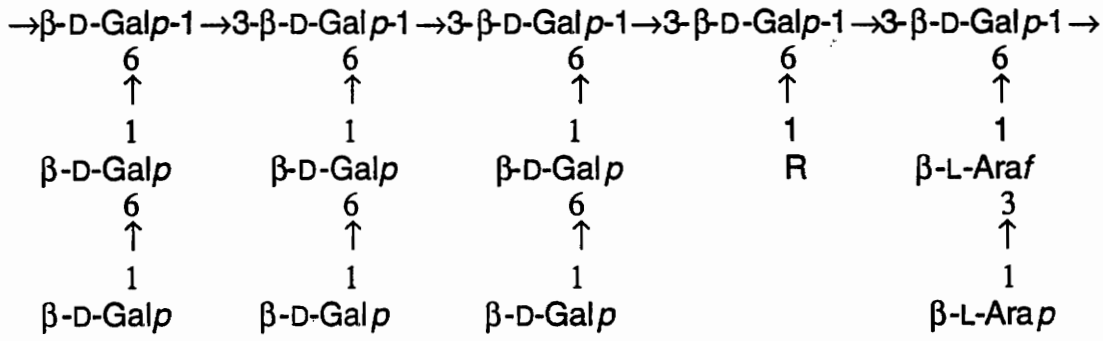


Figure 3. Representative structures of larch arabinogalactan (after Timell 1967).

The ratio of galactose to arabinose is about 6:1 in the arabinogalactan. The backbone of the arabinogalactan is composed of (1→3)-linked β-D-galactopyranose residues. Almost all galactose units on the backbone carry a side chain at their 6-positions. Most of these side

chains consist of (1→6)-linked β-D-galactopyranose residues, with two such units present per average chain. Some of the galactose units on the backbone also contain 3-*O*-β-L-arabinopyranosyl-L-arabinofuranose branches. Softwoods other than larches contain small amounts of arabinogalactans. Arabinogalactans isolated from several pines, spruces and Douglas fir have the same basic structures although the ratio of galactose to arabinose in the arabinogalactans might be different (Timell 1964). One difference between larch arabinogalactan and the others is that a larger portion of arabinose in arabinogalactans isolated from softwood species other than larches occurs as L-arabinofuranose end group (Aspinall and Wood 1963). It is noteworthy that compression wood contains large amounts of galactans. The backbone of these galactans is a (1→4)-linked β-D-galactopyranose residue.

Besides galactoglucomannan, arabino-4-*O*-methylglucuronoxylan, and arabinogalactan, softwood also contain small amount of other polysaccharides. These polysaccharides are mainly composed of arabinose, galactose, glucuronic and galacturonic acids.

1.1.2.3 Hardwood Hemicelluloses

Hardwood hemicelluloses are mainly composed of glucuronoxylan and glucomannan. Glucuronoxylan is much more abundant than glucomannan. Depending on the hardwood species, about 15-30% of dry hardwood consists of glucuronoxylan. All hardwoods have been found to have the same type of glucuronoxylan (Timell 1964). A representative structure is presented in Figure 4.

Glucuronoxylan is composed of xylose and 4-*O*-methyl glucuronic acid, and is simply called xylan. Xylan has a homogeneous backbone linked by (1→4) β-D-xylopyranose residues. The 4-*O*-methyl α-D-glucopyranosiduronic acid is linked to the 2-position of

xylose units on xylan backbone via a (1→2) glycosidic bond. About ten xylose units have one 4-*O*-methyl glucuronic acid. The xylan backbone is also highly acetylated. About 70% of xylose units is acetylated at C-3 or C-2 positions. Acetyl groups are easily hydrolyzed by alkali. The glycosidic bond between xylose units on the backbone is easily hydrolyzed by acid, whereas the linkage between xylose and 4-*O*-methyl glucuronic acid is very resistant to acid hydrolysis.

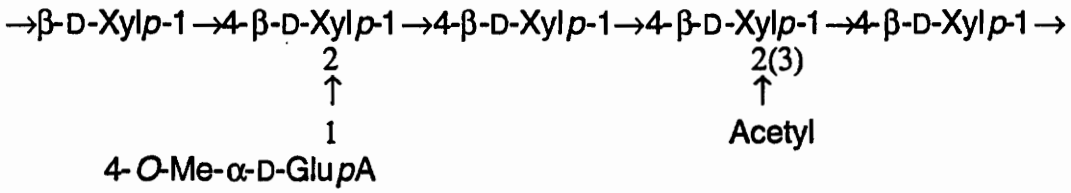


Figure 4. Representative structure of hardwood 4-*O*-methylglucuronoxylan (after Timell 1967).

Glucomannan is another hemicellulose in hardwoods. About 2-5% of hardwoods consists of glucomannan. Glucomannan is composed of β-D-glucopyranose and β-D-mannopyranose units linked by (1→4) glycosidic bonds. Depending on the hardwood species, the ratio of glucose to mannose varies between 1:1 and 1:2. In addition to glucuronoxylan and glucomannan, small amounts of miscellaneous polysaccharides are present in hardwoods. These polysaccharides are analogous to those present in softwoods. These polysaccharides may be important in the plant cell wall.

1.1.3 Lignin

Lignin is an amorphous, polyphenolic polymer. Lignin is widely distributed in plant kingdom, acting as a cementing component to connect cells and strengthen the xylem tissues. Lignin can be found in nature composed of one or a mixture of two to three

phenylpropane monomers: guaiacylpropane, syringylpropane and *p*-hydroxylphenylpropane. The composition of these three basic components in lignin structure varies with plant species. Softwoods are composed of mainly guaiacylpropane, whereas hardwood lignin is composed of approximately equal amounts of guaiacylpropane and syringylpropane units. Grass lignin is composed of guaiacylpropane, syringylpropane and *p*-hydroxylphenylpropane. Adler (1977) demonstrated that guaiacylpropane units are linked by both ether and carbon-carbon bonds. The major interunit linkages in softwood lignin are presented in Figure 5. The most abundant interunit linkage is the

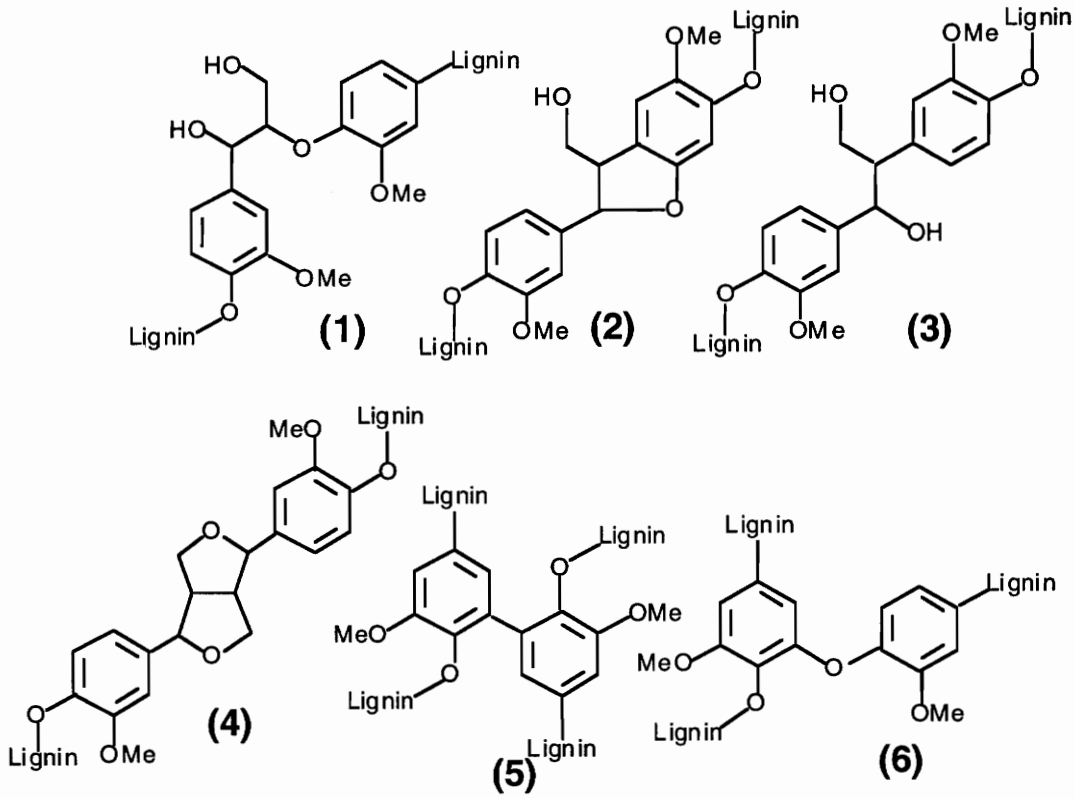


Figure 5. Major interunit linkages of guaiacyl lignin

guaiacylglycerol-β-guaiacyl ether (**1**), which accounts for 40-60% of all interunit linkages in softwood lignin. The other major interunit linkages are phenylcoumaran (**2**) (10%),

diarylpropane (**3**) (5-10%), pinoresinol (**4**) (< 5%), biphenyl (**5**) (5-10%), diphenyl ether (**6**) (5%). The interunit linkages in hardwood lignin and grass lignin are similar to those found in softwood lignin.

1.2 Biosynthesis of lignin, lignans and neolignans

1.2.1 Biosynthesis of lignin

Biosynthesis of lignin starts with the fixation of carbon dioxide in plants to form D-glucose. Glucose is further metabolized to tyrosine and phenylalanine via the shikimic acid

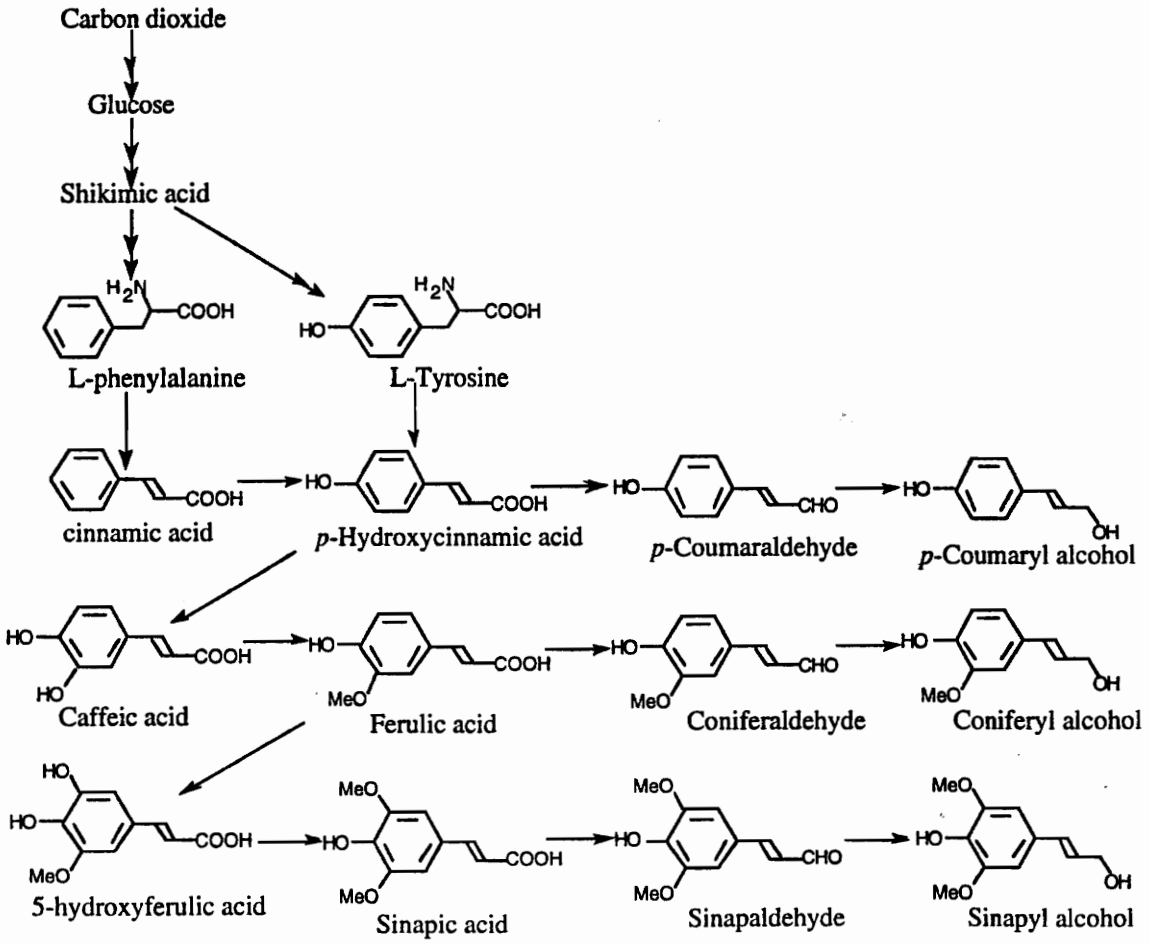


Figure 6. Biosynthetic pathway from carbon dioxide to lignin precursors

pathway (Higuchi *et al.* 1977, Higuchi 1990). Biosynthesis of lignin precursors starting from carbon dioxide is presented in Figure 6. The elimination of ammonia from the resulting tyrosine and phenylalanine provides *p*-hydroxycinnamic acid and cinnamic acid, respectively (Koukol and Conn 1961). These acids are further converted to ferulic acid via caffeic acid. The resulting ferulic acid can be further converted to sinapic acid by hydroxylation and subsequent methylation. In gymnosperms, *O*-methyltransferases (OMT) are essentially monofunctional and preferentially catalyze the formation of ferulic acid which is used directly for the biosynthesis of guaiacyl lignin. In angiosperms, OMT is a difunctional enzyme which catalyzes the formation of both ferulic acid and sinapic acid from caffeate and 5-hydroxylferulate, and is in favor of forming syringyl lignin. The sinapic, ferulic, and *p*-hydroxycinnamic acids are enzymatically reduced to the lignin precursors sinapyl, coniferyl, and *p*-coumaryl alcohols, respectively, via their corresponding aldehydes (Higuchi 1990). Kutsuki *et al.* (1982) has demonstrated that angiosperm enzymes reduce both coniferyl and sinapyl aldehydes to the corresponding alcohols almost equally, but the gymnosperm enzymes were remarkably specific for the reduction of coniferylaldehyde. It is believed that these *p*-hydroxylcinnamyl alcohol oxidoreductase and *O*-methyltransferases control the formation of guaiacyl and syringyl lignins (Higuchi 1990).

The last step to form lignin is the oxidative coupling of the lignin monomers into polymeric lignin. The monolignols are enzymatically oxidized into phenoxy free radicals which have several resonance structures (Figure 7) (Freudenberg 1968).

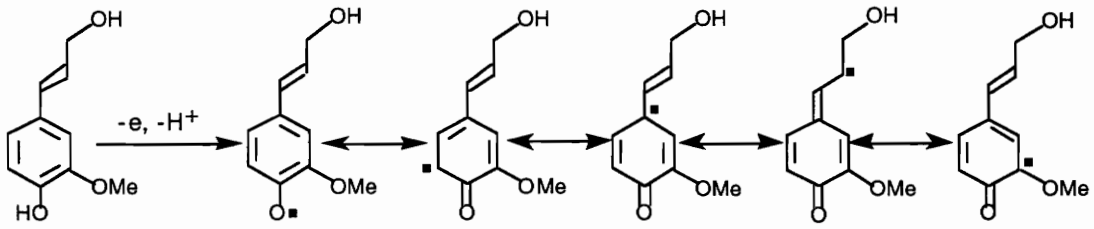


Figure 7. Enymatic dehydrogenation of coniferyl alcohol yielding phenoxy redicals

The random coupling of these resonance-stablized phenoxy radicals generates a variety of di- and oligolignols. The further oxidative coupling of these di and oligolignols provides polymeric lignin. Which enzymes are actually involved in the generation of phenoxy free radicals has been the subject of numerous investigations. Cousin and Hérissey (1908) first demonstrated that extracts from the fungus, *Russula delica*, can polymerize isoeugenol. Erdtman (1933) later proposed the hydrogenation theory of lignin biosynthesis through further investigation of isoeugenol polymerization, *i.e.* electrons generated from the oxidation of monolignols reduce oxygen to water. Because monolignols were not available at that time, the first synthetic lignin or DHP (dehydrogenation polymer) was accomplished by Freudenberg (1952). Freudenberg (1952) found that coniferyl alcohol was dehydrogenatively polymerized by a crude enzyme preparation from a mushroom (*Psalliota campestris*) under aeration. They later found that it was a laccase in the fungal extracts that catalyzed the polymerization. A laccase-like activity in cambial extracts from the gymnosperm *Araucaria excelsa* could also catalyze a similar polymerization (Freudenberg *et al.* 1958). However, peroxidases in these fungal and cambial extracts also catalyze the same polymerization when hydrogen peroxide is added (Freudenberg *et al.* 1958, Higuchi 1957, Higuchi and Ito 1958). Freudenberg (1959), however, did find an exception from Norway spruce (*Picea abies*) where peroxidases in cambial extracts would not catalyze the polymerization process. Higuchi and Ito (1958) found that peroxidases/H_2O_2 are widely distributed in higher plants, and can catalyze the polymerization of coniferyl alcohol to provide a DHP whose chemical properties are similar to that generated from the polymerization of coniferyl alcohol by laccase/O_2. Peroxidases/H_2O_2 appeared to generate DHPs more rapidly than laccase/O_2. Laccases were found to have limited distribution in higher plants (Higuchi 1990). Furthermore, Nakamura (1967) reported that a purified plant laccase from Japanese lacquer did not catalyze the polymerization of coniferyl alcohol, but a pure bamboo shoot peroxidase oxidized coniferyl alcohol into DHP. Thus it has been proposed that peroxidase but not

laccase is actually involved in catalyzing the polymerization of monolignols in higher plants. This hypothesis was further supported when Harkin and Obst (1973a) demonstrated that sections of tree stem could not oxidize syringaldazine without the addition of H_2O_2. Syringaldazine was used as a substrate to screen wood-rotting fungi for secreted peroxidases and phenoloxidases (Harkin and Obst 1973b). Peroxidase was widely accepted as the exclusive phenol oxidase responsible for the dehydrogenative polymerization of monolignols until some new studies with laccase were reported. Driouich *et al.* (1992) found that a laccase exists exclusively in sycamore maple cell walls, and is localized primarily in the xylem and epidermal stem tissues. Sterjiades *et al.* (1992, 1993) further demonstrated that this same laccase can catalyze the polymerization of monolignols to provide DHPs having structures identical to those generated by peroxidases. Bao *et al.* (1993) also found that a laccase isolated from cell walls of differentiating xylem of loblolly pine coincide in time and place with lignin formation, and can oxidize monolignols into DHP *in vitro*. The other studies also showed that laccases or laccase-like activities are closely correlated to the deposition of lignin in developing xylem (Davin *et al.* 1992, Savidge and Udagama-Randeniya 1992). These results suggest that laccase participates in lignin biosynthesis, and it seems that both laccases and peroxidases are involved. Sterjiades *et al.* (1993) found that laccases and peroxidases have different capabilities of polymerizing lignin model compounds. Therefore, Dean and Eriksson (1994) suggested that under some circumstances laccases may be primarily responsible for the initial polymerization of monolignols into oligolignols, whereas peroxidases continue to oxidize oligolignols into lignin macromolecules.

1.2.2. Biosynthesis of lignans and neolignans

Lignans and neolignans are a widely distributed, structurally diverse class of phenylpropanoids. Most of them are optically active, and exhibit some form of biological

activity (Davin and Lewis 1992, MacRae and Towers 1984). Lignans and neolignans are often implicated in natural plant defense and the decay resistance of certain woody tissues (heartwood, knots, *etc.*) (Anderegg and Rowe 1974). Lignans and neolignans also display important pharmacological properties in mammalian systems (Nikaido *et al.* 1981, Nishibe *et al.* 1990, Schröder *et al.* 1990, Richter *et al.* 1987). Although lignans and neolignans are an important and abundant group of natural products, little is known about their biosynthetic pathway, biosynthetic sites and their accumulation. Because lignans and neolignans are structurally analogous to the interunit linkages in lignin, they have been proposed to serve as the precursors of lignin (Rahman *et al.* 1990). The formation of lignans and neolignans has been speculated to proceed in a manner analogous to the enzymatic-catalyzed oxidative coupling of monolignols in the biosynthesis of lignin (Dewick 1990). However the biosynthesis of optically active lignans and neolignans obviously can not be explained by a random free radical coupling mechanism because oxidative coupling of monolignols by peroxidase/H_2O_2 or laccase/O_2 in the biosynthesis of lignin provides racemic products. The formation of optically active lignans and neolignans is somehow enzyme-mediated. It has been found that (-)-secoisolariciresinol and (-)-matairesinol occur in *Forsythia intermedia* , and not their corresponding enantiomers. (Umezawa *et al.* 1991). The biosynthesis of lignans in *Forsythia intermedia* has been found to be a highly enantioselective process (Figure 8).

Umezawa *et al.* (1990) first demonstrated that only radiolabelled (-)-secoisolariciresinol **10** and (-)-matairesinol **11** were isolated when [2-^{14}C] coniferyl alcohol **7** was administered to *F. intermedia* shoots for 3 hours; the corresponding radiolabelled enantiomeric forms were not detected. This implies that the formation of (-)-secoisolariciresinol **10** and (-)-matairesinol **11** is a highly stereoselective process. Further investigations have been directed to find the enzymes that are responsible for this stereoselectivity. Soluble cell-free extracts from *F. intermedia* can convert coniferyl

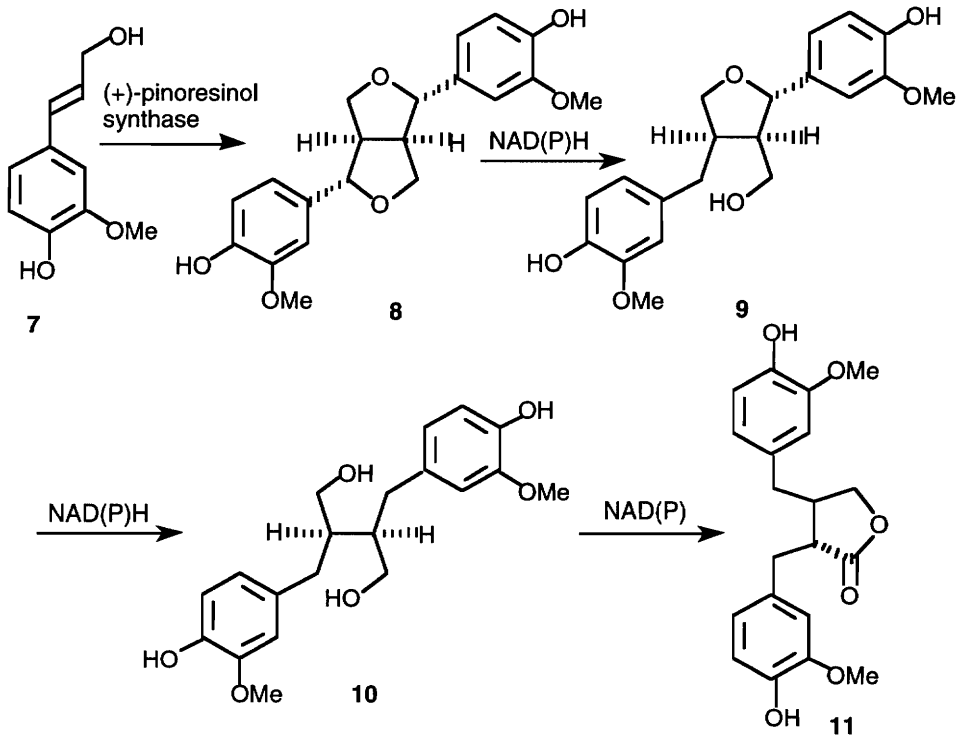


Figure 8. Biosynthesis of lignans in *F. intermedia*

alcohol into (-)-secoisolariciresinol **10** in the presence of cofactors NAD(P)H and H_2O_2 (Umezawa *et al.* 1990, 1991). During this experiment, an extraordinary accumulation of (-)-pinoresinol was observed. The racemic (±)-pinoresinols, but no (-)-secoisolariciresinol **10**, were found when coniferyl alcohol was incubated in the *F. intermedia* cell free preparations in the presence of H_2O_2. However, only (+)-pinoresinol **8** was consumed to provide (-)-secoisolariciresinol **10** when (±)-pinoresinol was incubated in the *F. intermedia* cell free preparations in the presence of NAD(P)H. It was later demonstrated that (-)-secoisolariciresinol **10** was synthesized from (+)-pinoresinol via (+)-lariciresinol **9** in *F. intermedia* cell free preparations (Katayama *et al.* 1992, 1993; Chu *et al.* 1993). The enantioselective NAD(P)H-dependent benzylic ether reductions of (+)-pinoresinol provide (+)-lariciresinol **9**. The further benzylic ether reduction of (+)-lariciresinol **9**

provides (-)-secoisolariciresinol **10** (Katayama 1993). The enzymatic dehydrogenation of (-)-secoisolariciresinol **10** affords (-)-matairesinol **11** (Umezawa *et al.* 1990b, 1991). Thus (+)-pinoresinol **8** is a key intermediate in the biosynthesis of lignans in *F. intermedia.* Because (-)-pinoresinol was not found in *F. intermedia*, and selective depletion of (-)-pinoresinol from the racemic (±)-pinoresinols was unlikely (Katayama *et al.* 1992), the formation of (+)-pinoresinol **8** is obviously not from the non-selective oxidative coupling of coniferyl alcohol with peroxidase/H_2O_2. Paré *et al.* (1994) isolated a (+)-pinoresinol synthase from *Forsythia intermedia.* The (+)-pinoresinol synthase is a one-electron oxidase which can catalyze the formation of (+)-pinoresinol (> 97% ee) from coniferyl alcohol. The (+)-pinoresinol synthase is the first isolated enzyme that can catalyze a stereoselective oxidative coupling of coniferyl alcohol. This is important progress in the biosynthesis of lignans and neolignans, and implies that free radical coupling of lignin precursors can be a highly stereoselective, not just a random coupling. The (+)-pinoresinol synthase has high substrate specificity. For example, the (+)-pinoresinol synthase cannot catalyze the enantioselective synthesis of syringaresinol. Biosynthesis of lignans in *F. intermedia* is the first example that biosynthesis of lignans is a highly enantioselective, enzyme-controlled process. The biosynthesis of (+)-pinoresinol in *Forsythia suspensa* has also been investigated (Umezawa *et al.* 1990, 1991; Davin *et al.* 1992).

As mentioned previously, lignans and neolignans have a very broad structural variation. Different lignans and neolignans are expected to have different biosynthetic pathways. The biosynthesis of neolignans and biogenetic differences among lignin, lignans and neolignans are still unsolved mysteries.

1.3 The mechanism of lignin-carbohydrate bond formation

According to the biosynthesis of lignin, lignin formation affords quinone methides as intermediates. Quinone methides are somewhat unstable and can undergo nucleophilic attack by various nucleophiles such as water, lignin hydroxyls, hydroxyls in carbohydrates, carboxylic acids. Freudenberg (1968) first demonstrated that dehydrogenative polymerization of coniferyl alcohol in the presence of sucrose afforded lignin-carbohydrate complexes. Carbohydrate was proposed to link to the α-position of the phenylpropane sidechain in lignin. Since then, the reaction of carbohydrates with quinone methide has been studied in detail (Sipilä and Brunow 1991a, b, Tanaka *et al.* 1976, 1979). Tanaka *et al.* (1976) found that the quinone methide reacted with D-glucose and methyl-D-glucose at almost equal rates, but the reaction of the quinone methide with 2,3,4,6-tetra-*O*-methyl-D-glucose was very slow. This implies that the C-1 hydroxyl is not the major reactive site in the addition of carbohydrates to the quinone methide. They also found that lignin was mainly linked to the primary hydroxyl of D-glucose and methyl D-glucose. The reason for this selectivity is that the primary hydroxyls are less sterically hindered than secondary hydroxyls. Tanaka *et al.* (1979) also demonstrated that the reaction of D-glucuronic acid with the quinone methide afforded ester-linked lignin-carbohydrate complexes, and D-glucuronic acid is more reactive than D-glucose. This is in accord with Freudenberg's observation that the reactivity of nucleophiles to quinone methide depends on the acidity of the nucleophile (Freudenberg 1968).

In addition to above quinone methide addition mechanism, carbohydrates may also be linked to lignin by biochemical processes. For example, glucose transferase is widely distributed in plant cell walls which include the lignifying cell wall. Glucose in UDP-glucose can be glucosidically linked to the primary hydroxyl of coniferyl alcohol to provide isoconiferin (Ibrahim 1977). The dehydrogenative polymerization of the resulting isoconiferin would provide LCC having glucose residues linked to the primary hydroxyl of the propane sidechain of lignin via a glycosidic bond. Glycosidic linkages between

carbohydrates and the primary hydroxyls in dilignols have been found in the extractives of conifers (Theander 1977). Joseleau and Kesraoui (1986) demonstrated that glycosidic linkages between the monosaccharides and the polyphenol were formed when dehydrogenative polymerization of coniferyl alcohol with a crude enzyme extract from *Populus tremula* was carried out in the presence of arabinose and glucose. The transglycosylation reaction of *Lyophyllum ulmarium* β-xylanase in the presence of various lignin model compounds has been investigated (Amano *et al.* 1993). Several xyloside-linked lignin-carbohydrate model compounds have been isolated. It is possible that transglycosylation occurs on all hydroxyl groups of oligolignols and lignin macromolecules. Therefore, the possibility that glycosidic linkages between lignin and carbohydrates exist cannot be eliminated.

Ferulic acid is the precursor of monolignol coniferyl alcohol. Ferulic acid is present during the lignification of the grass cell wall. Ferulic acid is mainly esterified in grasses with hemicelluloses (Scalbert *et al.* 1985; Atsushi *et al.* 1984). It has been demonstrated that methyl 5-*O*-(*E*)-feruloyl-α-L-arabinofuranoside (FA-Ara) participated in the free radical coupling during the dehydrogenative polymerization of coniferyl alcohol (Ralph *et al.* 1992). Interunit linkages between FA-Ara and coniferyl alcohol are analogous to the interunit linkages in lignin. Lignin carbohydrate linkages may be formed by this copolymerization. Moreover, ferulic acid is bifunctional. It is possible that the carboxyl group of ferulic acid is esterified with hemicelluloses; the phenolic group of ferulic acid is linked to lignin via an ether bond. Ferulic acid serves as a bridge between lignin macromolecules and grass hemicelluloses.

1.6 Linkages between lignin and hemicellulose

According to the potential mechanisms of forming lignin-carbohydrate (LC) linkages, the

following types of LC linkages may exist in plants.

1) Ester linkages between lignin and uronic acids or between hemicelluloses and phenolic acids.

2) Ether linkages between lignin and hemicellulose hydroxyls.

3) Glycosidic linkages between lignin and hemicelluloses.

Enormous efforts have been expended to characterize the linkages between lignin and hemicelluloses. In terms of indirect evidences gathered from the investigations of LCCs isolated from softwood and hardwood species, specific linkage sites between lignin and hemicelluloses have been proposed. The proposed LC linkages are reviewed below.

More efforts have been directed to characterize LC linkages in softwoods than hardwoods. Watanabe *et al.* (1989) studied the LC linkage sites in the neutral fraction of a pine LCC by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) method. They found that mannose, galactose and glucose in galactoglucomannan are *O*-6 ether linked to lignin; xylose in arabinoglucuronoxylan is *O*-2 and *O*-3 ether linked to benzyl positions of lignin. Watanabe and Koshijima (1988) also demonstrated that 4-*O*-methylglucuronic acid residue in arabinoglucuronoxylan is bonded to lignin by an ester linkage in *Pinus densiflora* wood. Obst (1982) demonstrated that alkali-labile LC linkages exist in milled wood enzyme lignin (MWELs) isolated from Loblolly pine, Sitka spruce, Red pine and Aspen. Of these alkali-labile linkages, an ester linkage between glucuronic acid in xylan and lignin was proposed to be the predominant bond. The author also studied LC bond frequencies. Hardwood and softwood MWELs were found to have approximately equal LC bond frequencies, but MWEL generated from compression wood contained more LC bonds than normal wood. Most of LC linkages in borohydride-reduced MWEL prepared from loblolly pine were stable in soda pulping conditions. Results from methylation analysis of LCCs isolated from loblolly pine (Minor 1982) indicated that *O*-6 positions of glucose, galactose and mannose are preferentially linked to lignin; the arabinose is bonded to lignin

exclusively at *O*-5 position. Using the DDQ method to investigate LCCs isolated from pine (*Pinus densiflora*) wood, Takahashi and Koshijima (1988b) found that lignin is linked to xylose at the C-2 or C-3 positions through an alkali-labile benzyl ether bond. Eriksson and Lindgren (1977) used a cellulase-hemicellulase mixture to digest spruce LCC. The authors found that arabinoglucuronoxylan is linked to lignin via arabinose side chain and xylose units on the backbone; galactoglucomannan is linked to lignin through the galactose side chain. Using the Smith degradation method, Yaku *et al.* (1981) demonstrated that *O*-2 or *O*-3 positions of mannose and glucose in a neutral fraction of Björkman LCC isolated from *Pinus densiflora* are linked to lignin. Eriksson and Goring (1980) investigated LCCs isolated from Black spruce (*Picea mariana*) by using hemicellulase hydrolysis, alkaline hydrolysis, acidic hydrolysis and borohydride reduction. They found that arabinoglucomannan is ether bonded to lignin at C-2 or C-3 positions of arabinose and xylose units. Lignin is also linked to 4-*O*-methyl glucuronic acid in arabinoglucomannan by an ester bond. For the galactoglucomannan, C-3 position of the galactose units is linked to lignin. They also suggested that cellulose is linked to lignin. Iverson and Wännström (1986) suggested that base stable LC ether bonds were formed during the kraft pulping of pine wood. Gierer and Wännström (1984) demonstrated that C-C bonds were formed between lignin and carbohydrate fragments during kraft pulping of spruce wood (*Picea abies*). Using DDQ and borohydride reduction, combined with acid and base hydrolysis methods, Kosíková *et al.*(1979) found that phenolic benzyl ethers are unstable in the alkaline medium, whereas non-phenolic benzyl ether bonds are stable to alkaline hydrolysis. The authors estimated that frequency of lignin-lignin bonds were two to eight times more than that of the lignin-carbohydrate linkages in LCCs isolated from spruce wood. Kosíková and Ebringerová (1994) demonstrated that both glycosidic and ether types of bonds exist between residual lignin and cellulose in pulp generated from soda pulping of spruce wood. Johnson and Overend (1992) investigated the effect of hydrolyzing enzymes on the enzymatic hydrolysis of

deltoides. They found that polysaccharides were not completely hydrolyzed when the LCC was treated with various combinations of acetyl xylan esterase, xylanase, β-mannanase, and β-glucosidase. In most cases, the effect of different enzymes was not additive. The limited polysaccharide hydrolysis was believed to result from conformational changes which limit enzyme action.

Lignin carbohydrate linkages in hardwood species have also been investigated. Using alkaline hydrolysis and Smith degradation methods, Azuma *et al.* (1985) demonstrated the presence of alkaline-labile and alkaline-stable linkages between lignin and carbohydrates in LCC isolated from Beech wood (*Fagus crenata* BI.). Takahashi and Koshijima (1988a,b) investigated LC linkages in Beech (*Fagus crenata*) wood by using DDQ, borohydride reduction methods. They found that lignin is linked to glucuronoxylan at the C-2 or C-3 positions of xylose units by ether bonds, at 4-*O*-methyl glucuronic acid by an ester bond. They also determined that about 30% of the glucuronic acid present in LCC was involved in this ester linkage. The lignin portion of the LCCs consisted of a small number of molecules with a very large molecular weight. Watanabe *et al.* (1992) demonstrated that glucuronoxylan in LCC isolated from the tropical hardwood *Albizia falcata* (L.) Back was directly bound to lignin through C-2 and C-3 positions of xylose unit by ether linkages. Using an improved procedure of DDQ oxidation, Imamura *et al.* (1994) demonstrated that 4-*O*-methyl glucuronic acid in glucuronoxylan is linked to lignin by an ester bond. They proposed that 4-O-methyl glucuronic acid is linked to α-position of the lignin side chain. We (Li and Helm 1995) have observed that 4-*O*-methyl glucuronosyl group migrated between α- and γ-positions of the lignin side chain in both neutral and acidic conditions. This observation implies that the classical view of lignin-xylan ester linkages in wood needs to be reevaluated.

All lignin-carbohydrate covalent linkages reported so far are proposed according to indirect evidence. From the results obtained, it is clear that lignin is covalently linked to hemicellulose. However, the nature of these LC linkages are still poorly understood. In particular, the amount, distributions and functions of these LC bonds are basically unknown. Further investigations must be conducted in order to better utilize wood and fiber.

1.5 References

Adler, E., *Wood Sci. Technol.*, 11 (**1977**) 169.

Amano, Y.; Fujita, I; Matsuzawa, T.; Okazaki, M. and Kanda, T., *Mokuzai Gakkaishi*, 39 (**1993**) 814.

Anderegg, R. J. and Rowe, J. W., *Holzforschung*, 28 (**1974**) 171.

Aspinall, G. O. and Wood, T. M., *J. Chem. Soc.* (**1963**) 1686.

Atalla, R. H. and van der Hart, D. L., *Science*, 223 (**1984**) 283.

Atsushi, K.; Azuma, J. I. and Koshijima, T., *Holzforchung*, 38 (**1984**) 141.

Azuma, J.-I.; Takahashi, N.; Isaka, M. and Koshijima, T., *Mokuzai Gakkaishi*, 31 (**1985**) 587.

Bao, W.; O'Malley, D. M.; Whetten, R. and Sederoff, R. R., *Science*, 260 (**1993**) 672.

Blackwell, J., *The macromolecular organization of cellulose and chitin.* In *Cellulose and other natural polymer systems: biogenesis, structure and degradation.* Brown R. M. Jr, Ed., Plenum, New York, **1982**, 403.

Chu, A.; Dinkova, A.; Davin, L. B.; Bedgar, D. L. and Lewis, N.G., *J. Biol. Chem.*, 268 (**1993**) 27026.

Cousin, H. and Hérissey. H., *C.R.A.S., Paris*, 147 (**1908**) 247.

Davin. L. B. and Lewis, N. G. in *Recent Advances in Phytochemistry*, Stassord, H. A. and Ibrahim, R. K., Eds., Plenum Press, New York, vol 26, **1992**, 325.

Davin, L. B.; Bedgar, D. L.; Katayama, T. and Lewis, N. G., *Phytochemistry*, 31 (**1992**) 3869.

Dean, J. F. D. and Eriksson, K-E. L., *Holzforschung*, 48 (**1994**) Suppl. 21.

Dewick, P. M. in *Studies in Natural Products Chemistry. Vol. 5. Structure Elucidation.* Part B/Atta-ur-Rahman, Ed., Elservier, Amsterdam, **1990**, 459.

Driouich, A.; Lainé, A. C.; Vian, B. and Faye, L. *Plant J.*, 2 (**1992**) 13.

Erdtman, H., *Biochem. Z.*, 258 (**1933**) 172.

Eriksson, O. and Lindgren, B. O., *Svensk Papperstidn.*, 2 (**1977**) 59.

Eriksson, O and Goring, D. A. I, *Wood Sci. Technol.*, 14 (**1980**) 267.

Fengel, D. and Wegner, D., *Wood Chemistry, Ultrastructure, Reactions*. Walter de Gruyter, Berlin, New York, **1989**.

Freudenberg, K., *The constitution and biosynthesis of lignin.* In *Constitution and biosyntheses of lignin.* Freudenberg, K. and Neish A. C. (Eds.) Spring-Verlag, Berlin, **1968**, 45.

Freudenberg, K., *Holzforschung*, 6 (**1952**) 37.

Freudenberg, K.; Harkin, J. M.; Rechert, M. and Fukuzumi, T., *Chem. Ber.*, 91 (**1958**) 581.

Freudenberg, K., *Nature*, 183 (**1959**) 1152.

Gardener, K. H. and Blackwell, J., *Biopolymers*, 13 (**1974**) 1975.

Gierer, J. and Wännström, S., *Holzforschung*, 38 (**1984**) 181.

Harkin, J. M. and Obst, J. R., *Science*, 180 (**1973a**) 296.

Harkin, J. M. and Obst, J. R., *Experentia*, 29 (**1973b**) 381.

Higuchi, T., *Physiol. Plant.*, 10 (**1957**) 356.

Higuchi, T.; Shimada, M.; Nakatsubo, F. and Tanahashi, M., *Wood Sci. Technol.*, 11 (**1977**) 153.

Higuchi, T., *Wood Sci. Technol.*, 24 (**1990**) 23.

Higuchi, T. and Ito, Y. *J. Biochem.* 45 (**1958**) 575.

Ibrahim, P. K., *Z. Pflanzenphysiol.*, 85 (**1977**) 253.

Iversen, T. and Wännström S., *Holzforschung*, 40 (**1986**) 19.

Johnson, K. G. and Overrend, R. P., *Holzforchung*, 46 (**1992**) 31.

Jones, J. K. N. and Painter, T. J., *J. Chem. Soc.*, (**1957**) 669.

Jones, J. K. N. and Painter, T. J., *J. Chem. Soc.* (**1959**) 573.

Joseleau, J. P. and Ganet, C., *Svensk. Papperstidn.* 84 (**1981**) 123.

Joseleau, J.-P. and Kesraoui, R., *Holzforschung*, 40 (**1986**) 163.

Katayama, T.; Davin, L. B. and Lewis, N. G., *Phytochemistry*, 31 (**1992**) 3875.

Katayama, T.; Davin, L. B.; Chu, A. and Lewis, N. G., *Phytochemistry*, 33 (**1993**) 581.

Kitagawa, S.; Nishibe, S.; Benecke, R. and Thieme, H., *Chem. Pharm. Bull.*, 36 (**1988**) 3667.

Koshijima, T., *Nippon Mokuzai Gakkaishi* , 6 (**1960**) 194.

Kosíková, B. and Ebringerová, A., *Wood Sci. Technol.*, 28 (**1994**) 291.

Koukol, J. and Conn. E. E., *J. Biol. Chem.*, 236 (**1961**) 2692.

Kutsuki, H.; Shimada, M. and Higuchi, T., *Phytochemistry*, 21 (**1982**) 19.

MacRae, W. D. and Towers, G. H. N., *Phytochemistry*, 23 (**1984**) 1207.

Meier, H., *Acta Chem. Scand.*, 15 (**1961**) 1381.

Nakamura, W., *J. Biochem.*, 62 (**1967**) 54.

Marchessault, R. H.; Holava, H. and Timell, T. E., *Can J. Chem.*, 41 (**1963**) 1612.

Nikaido, T.; Ohmoto, T.; Kinoshita, T., Sankawa, U., Nishibe, S. and Hisada, S., *Chem. Pharm. Bull.*, 29 (**1981**) 3586.

Nishibe, S., Kinoshita, H., Takeda, H. and Okano, G., *Chem. Pharm. Bull.*, 38 (**1990**) 1763.

Obst, J. R., *Tappi*, 65 (**1982**) 109.

Paré, P. W.; Wang, H. B., Davin, L. B. and Lewis, N. G., *Tetrahedron Lett.*, 35 (**1994**) 4731.

Rahmanm M. M. A.; Dewick, P. M.; Jackson, D. E. and Lucas, J. E., *Phytochemistry*, 29 (**1990**) 1841.

Ralph, J.; Helm, R. F.; Quideau, S. and Hatfield, R. D., *J. Chem. Soc. Perkin Trans. 1*, (**1992**) 2961.

Richter, A.; Strausfeld, U. and Knippers, R., *Nucleic Acids Res.*, 15 (**1987**) 3455.

Savidge, R. and Ugagama-Randeniya, P., *Phytochemistry*, 31 (**1992**) 2959.

Scalbert, A.; Monties, B.; Lallemand, J. Y.; Guittet, E. and Rolando, C., *Phytochem.*, 24 (**1985**) 1359.

Schröder, H. C.; Merz, H.; Steddenm R.; Muller, W.E.G.; Sarin, P. S.; Trumm, S.; Schulz, J. and Eich, E., *Z. Naturforsch.*, 45c (**1990**) 1215.

Sipilä, J. and Brunow, G., *Holzforshung*, 45 (**1991a**) suppl. 3.

Sipilä, J. and Brunow, G., *Holzforshung*, 45 (**1991b**) suppl. 9.

Sterjiades, R.; Dean, J. F. D. and Eriksson, K-E. L., *Plant Physiol.*, 99 (**1992**) 1162.

Sterjiades, R.; Dean, J. F. D.; Gamble, G.; Himmelsbach, D. S.and Eriksson, K-E. L., *Planta.*, 190 (**1993**) 75.

Takahashi, N. and Koshijima, T., *Wood Sci. Technol.*, 22 (**1988a**) 231.

Takahashi, N. and Koshijima, T., *Wood Sci. Technol.*, 22 (**1988b**) 177.

Tanaka, K.; Nakatsubo, F. and Higuchi, T., *Mokuzai Gakkaishi* , 22 (**1976**) 589.

Tanaka, K.; Nakatsubo, F. and Higuchi, T., *Mokuzai Gakkaishi*, 25 (**1979**) 653.

Theander, O. *Tappi Conference papers, Forest Biology/Wood Chemistry Conference* , **1977**, 37.

Timell, T. W., *Adv. Carbohydr. Chem.*, 19 (**1964**) 247.

Timell, T. W., *Adv. Carbohydr. Chem.*, 20 (**1965**) 409.

Timell, T. W., *Wood Sci. Technol.*, 1 (**1967**) 45.

Tönnesen B. A. and Ellefsen, Ö, *Sub-microscopical investigations.* In *Cellulose and cellulose derivatives*, Bikales, N. M., Segal, L. Eds. Wiley, New York, Vol. 5, Part IV, (**1971**) 265.

Umezawa, T.; Davin, L.B.; Yamamoto, E.; Kingston, D. G. I. and Lewis, N. G., *J. Chem. Soc., Chem. Comm.* **1990**, 1405.

Umezawa, T.; Davin, L. B. and Lewis, N. G., *J. Biol. Chem.*, 266 (**1991**) 10210.

Vaughanm, J. M. and Dickey, E. E., *J. Org. Chem.*, 29 (**1964**) 715.

Watanabe, T. ; Ohnishi, J.; Yamasaki, Y.; Kaizu, S. and Koshijima, T., *Agric. Biol. Chem.*, 53 (**1989**) 2233.

Yaku, F.; Tanaka, R. and Koshijima, T., *Holzforschung*, 35 (**1981**) 177.

Chapter 2

Methods to Investigate Covalent Linkages in Wood

2.1 Introduction

Since Björkman (1957) suggested that lignin was chemically linked to carbohydrates, enormous efforts have been expended to prove the existence of covalent bonds between lignin and carbohydrates in plant cell walls. More and more evidence suggests that lignin and hemicellulose are intimately linked to one another through covalent bonds. Initially, the interest was focused on the occurrence of lignin-carbohydrate linkages. For example, studies have been directed at the formation of lignin-carbohydrate complexes during the dehydrogenative polymerization of lignin monomers in the presence of carbohydrates (Tanaka *et al.*, 1979; Katayama *et al.*, 1980; Ohara *et al.*, 1980; Joseleau and Kesraoui, 1986). The interest later changed to the determination of binding sites between lignin and hemicelluloses, and many methods have been developed for analysis of this interaction. The commonly used methods are chemical and enzymatic analysis of lignin-carbohydrate complexes (LCC). For example, exhaustive methylation of isolated LCCs, followed by acidic hydrolysis and analysis of the hydrolysate provided indirect evidence of the binding sites on hemicellulose (Minor, 1982, 1986). The other method utilizes 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). It was reported that DDQ could selectively cleave benzyl ethers and benzyl esters in LCCs (Watanabe, 1989). The use of these methods, especially DDQ, have significantly improved our understanding of lignin-carbohydrate linkages. However, these methods only provide indirect evidence, and have serious limitations. Low yield in the DDQ cleavage of some appropriate model compounds suggests that care must be taken in the interpretation of results from the DDQ method

(Sipilä *et al.*, 1996). It has long been a goal that the presence of lignin-carbohydrate covalent bonds can be directly determined by NMR spectroscopy. In order to achieve this goal, the establishment of an NMR database by the synthesis of model compounds is required. Moreover, the study of model compounds can provide information on the reactivity of LC linkages so that methods of either isolating LCCs without degradation or selectively cleaving LCCs can be established. This is referred to as the model compound method. The model compound method can provide information that cannot be gathered from native wood cell walls or their isolates due to the inherent complexity of wood cell walls. The methods of characterizing LC linkages will be reviewed here. Because the characterization of LC linkages cannot be accomplished without an appropriately isolated LCC, methods of isolating LCCs will be reviewed first.

2.2 Isolation of lignin-carbohydrate complexes (LCC)

Because lignin is believed to be covalently linked to hemicelluloses, methods of isolating LCCs begins with the isolation of lignin. The isolation of lignin has been extensively investigated in order to determine the structures of this natural product. Initially, acid lignin preparations (Klason lignin) were widely used in the chemical characterization of lignin structures. However, it was later found that the structure of lignin was extensively changed during the acid treatment (Lai and Sarkanen, 1971). Brauns (1939, 1952) isolated essentially unchanged lignin by direct extraction of wood with organic solvents. This lignin preparation is the so-called Brauns lignin. The studies on Brauns lignin have provided useful information on the structure of lignin. However, the yield of Brauns lignin is very low, and Brauns lignin has a low molecular weight and a high phenol content. It was found that a milling process before solvent extraction would increase the solubility of wood in organic solvents, whereas this milling process would not cause profound chemical modification in wood (Staudinger *et al.*, 1936). A modified method

was then introduced to isolate lignin, so-called milled wood lignin (MWL) or Björkman lignin (Björkman, 1956,1957; Björkman and Person, 1957). MWL was obtained by aqueous dioxane extraction of finely ground wood meal. The yield of MWL was much higher than that of Brauns lignin. MWL was (and still is) the most commonly used lignin preparation in the structural studies.

Once the finely milled wood meal has been obtained, methods to obtain LCCs differ in either direct extraction with solvents or the treatment with enzyme preparations. N, N-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were used in the extraction of the residual wood meal previously extracted with aqueous dioxane to provide LCCs (Björkman LCC) (Björkman, 1957). The lignin content of Björkman LCC is similar to MWL, but Björkman LCC has a higher carbohydrate content. (Azuma *et al.*, 1981). Björkman (1957) suggested that chemical linkages existed between lignin and carbohydrate because he could not separate carbohydrates from lignin without chemical degradation. Moreover, Yaku *et al.* (1976) reported that the hot water extraction of the residual wood meal after extraction of Björkman LCC resulted in approximately the same amount of LCC as Björkman LCC (termed HWF). A large number of studies on Björkman LCC or HWF have strongly suggested the existence of covalent linkages between lignin and carbohydrates.

Björkman LCC is widely used for the determination of lignin-carbohydrate linkages. However, it is troublesome to prepare in large quantities because complete removal of non-volatile organic solvents by dialysis is quite time-consuming. A convenient alternative to prepare LCCs was developed by Watanabe (Watanabe, 1989). The finely ground wood meal, previously extracted with 80% aqueous dioxane, was extracted with cold water and then with hot water to provide a water-soluble LCC. A large amount of this water-soluble LCC can be easily prepared in a short period of time. This LCC was further divided into

neutral, acidic and lignin-rich fractions by anion-exchange chromatography. These subfractions have chemical properties similar to Björkman LCCs. This method was recommended to replace the Björkman procedure in the preparation of LCCs (Watanabe, 1989).

During the studies on the above mentioned LCCs, it was found that high carbohydrate contents was a problem in the binding-site analysis of the LCCs. This is because the frequency of LC bonds is much lower than that of glycosidic bonds of carbohydrates. Therefore, a complete enzymatic hydrolysis of carbohydrates was investigated prior to the binding site analysis of LCCs. One way to prepare enzyme-degraded LCCs was to digest milled wood meal with cellulase and hemicellulases for removal of polysaccharides prior to LCC solvent extraction (Pew, 1957). This enzyme-degraded LCC is known as milled wood enzyme lignin (MWEL) or cellulase-treated enzyme lignin (CEL) (Pew, 1957). MWEL was proposed to contain nearly all the LC linkages present in wood as long as none are glycosidically linked (Obst, 1982). Aqueous dioxane extraction of residual wood meal previously digested with cellulase and hemicellulases provided much higher yield of lignin present in wood than MWL. More than half of the lignin could be dissolved in aqueous dioxane. MWL is believed to be an adequate representative of the total lignin, whereas MWEL is believed to be more suitable for the structural studies of lignin because MWEL is less altered than MWL. Furthermore, MWEL can be obtained in good yield with less degradation. Therefore, MWEL is good for both studies of lignin and LCC linkages. However, MWEL thus obtained contains high molecular weight lignin, which turns out to be an obstacle in the analysis of LC bonds. Attention was then turned to enzymatic hydrolysis of Björkman LCC and water-soluble LCCs. LCCs prepared in this manner have moderate lignin molecular weights. After polysaccharides are thoroughly digested away from the LCCs, frequency of LC bonds is significantly increased. A drawback for this enzymatic hydrolysis of Björkman LCCs and water-soluble LCCs is the

difficulty in isolating LCC fragments from the enzymatic hydrolyzates. Fortunately, the resulting LCCs have amphiphatic properties because the same LCC molecule contains hydrophilic carbohydrates and hydrophobic lignin. As a result, LCCs can be isolated by adsorption chromatography (Watanabe, 1989). It is noteworthy that enzymatic treatment of milled wood meal or isolated LCCs cannot completely digest the polysaccharides (Johnson and Overend, 1992). Two possibilities exist for this incomplete digestion. One possibility is that lignin in LCCs inhibit enzyme activity. The other possibility is that hydrolysis of different polysaccharides require different enzymes, and a mixture of enzymes might not be additive. Research on biodegradation of cellulose and hemicelluloses has been reviewed in detail (Eriksson *et al.*, 1990; Jeffries, 1994).

2.3 General chemical methods

2.3.1 Alkali degradation method

Many methods have been developed to indirectly prove the existence of lignin-carbohydrate covalent linkages. One of these methods utilizes alkali degradation (Wang *et al.* , 1967; Merewether and Samsuzzaman, 1972; Kosíková *et al.*, 1979; Smelstorius, 1974; Yaku *et al.*, 1976; Eriksson and Goring, 1980; Obst, 1982; Lundquist *et al.*, 1983; Takahashi and Koshijima, 1988a). The alkali degradation methods are mainly used to determine alkali-labile bonds in LCCs. It is well known that ester groups are labile to alkaline hydrolysis. However, an alkali-labile bond in LCCs does not necessarily have to be an ester. Kosíkavá *et al.* (1979) found that phenolic benzyl ether bonds were easily hydrolyzed in an alkaline solution; non-phenolic benzyl ether bonds were stable to alkaline hydrolysis. Enoki *et al.* (1983) also draw the same conclusion through the alkaline hydrolysis of model compounds.

The use of alkali in determining LC bonds is only useful when coupled with enzymatic hydrolysis, IR and UV spectroscopies, ion-exchange column chromatography, aqueous borohydride reduction. Milled wood enzyme lignin (MWEL) is believed to contain almost all of the original lignin in the wood and about 10-15% residual carbohydrates. These residual carbohydrates cannot be significantly removed by remilling, redigestion, and many other methods except acid hydrolysis (Pew, 1957). This would seem to suggest that the carbohydrates are covalently linked to lignin. It has been theorized that all LC bonds except glycosidic LC bonds should be present in MWEL because the enzyme mixture used for the preparation of MWEL also contains phenyl glycosidases (Obst, 1982). MWEL is insoluble in water but dissolves extensively in 0.1 N sodium hydroxide (Obst, 1982). Obst (1982) found that about 10 to 20% of residual carbohydrates in MWELs from loblolly pine, Sitka spruce, red pine, and aspen was removed after mild alkaline treatment. This provided indirect evidence for the presence of alkali-labile bonds in MWEL. Some research was directed to determine specific alkali-labile linkages and binding sites in LCCs isolated from both hardwood and softwood (Takahashi and Koshijima, 1988a, b). Takahashi and Koshijima (1988a) isolated a water soluble LCC from beech (*Fagus crenata*), and found that the IR absorbance in the vicinity of 1730 cm^{-1} due to esterified carbonyl groups disappeared after the water soluble LCC was treated with mild alkali. Because the UV spectrum showed that the original LCC did not contain any phenolic acids, the IR absorbance around 1730 cm^{-1} was proposed to be from acetyl groups and 4-*O*-methyl glucuronates. The elution profile of the alkali-treated LCC on Sephadex LH-60 clearly showed two new peaks for lignin and a new peak for carbohydrates while the original LCC had only a single peak eluting at the void volume. These results indicate that this LCC contained alkali-labile LC bonds such as esters. The phenolic hydroxyls in the water soluble LCC were methylated before mild alkaline hydrolysis so as to rule out the interference from phenolic benzyl ethers.

The presence of phenolic benzyl ether linkages between lignin and carbohydrates in an LCC isolated from pine wood has also been determined by the alkali degradation methods (Eriksson and Goring, 1980). Using alkaline hydrolysis, combined with Sephadex LH-60 chromatography and DDQ oxidation, Takahashi and Koshijima (1988b) concluded that lignin was covalently linked to the 2 and 3 positions of xylose through an alkali-labile benzyl ether bond in LCCs isolated from pine (*Pinus densiflora*) and beech (*Fagus crenata*).

2.3.2 Acid degradation method

Acid hydrolysis has been widely used in the structural determination of polysaccharides (Jansson *et al.*, 1976; Minor, 1982; Ford and Elliott, 1987; Neilson and Richards, 1982). Strong acidic hydrolysis of polysaccharides, and conversion of the hydrolysate into trimethylsilyl ether or alditol acetates are generally used to determine the monosaccharide composition of polysaccharides. Methylation of polysaccharides, followed by acid hydrolysis and the reduction of hydrolysate is usually used to determine the binding sites of sugar units. Minor (1982, 1986, 1991) used methylation and acid hydrolysis method to determine LC covalent linkages in LCCs isolated from loblolly pine wood and red pine compression wood. The presence of large amounts of 2,3-di-*O*-methyl-arabinose suggested that lignin was linked to arabinose at C-5 because arabinose units form non-reducing end groups in the hemicellulose molecules (Timell, 1967). That there was more 2-*O*-methyl-xylose than terminal arabinose also led him to conclude that lignin must be bonded to the C-3 position of xylopyranose. It has been demonstrated that furanosidic linkages can be selectively cleaved under mild acid conditions (Eriksson and Lindgren, 1977). Under mild acid conditions, the linkages between the arabinose side-chains and the xylopyranose backbone in the xylan molecules would be selectively cleaved without affecting the other acid sensitive bonds. The selective cleavage of arabinofuranosidic

linkage under mild acid conditions has made the mild acid hydrolysis a useful method in the investigations of LC linkages. Eriksson and Goring (1980) found that xylan fragments were removed to a larger extent than galactoglucomannan and cellulose fragments after the mild acid hydrolysis of a borohydride reduced LCC that was prepared from a commercial hemicellulase treatment of Björkman LCC isolated from black spruce (*Picea mariana*), followed by DMF extraction. This result led them conclude that arabinose side-chains were chemically linked to lignin.

2.3.3 Borohydride reduction

Aqueous sodium borohydride reduction has been used in determining LC bonds in LCCs (Kosíková *et al.*, 1979; Eriksson and Goring, 1980; Takahashi and Koshijima, 1988a,b). Sodium borohydride can reduce esters and lactones, but cannot reduce free uronic acid. The reactivity difference of sodium borohydride on esters and free uronic acids has been evaluated through some model compounds (Takahashi and Koshijima, 1988a). A detection of 4-*O*-methyl glucose from the LCC reduced by aqueous sodium borohydride is believed as an evidence of an ester linkage between lignin and carbohydrates. Comtat *et al.* (1974) first reported the isolation of 4-*O*-methyl glucose from a glucuronoxylan obtained from a reduced aspen holocellulose. However, the possibility that 4-*O*-methyl glucose was derived from a uronic acid lactone was not eliminated. After this possibility was eliminated, ester linkages between lignin and carbohydrates were proposed in a LCC isolated from beech (*Fagus crenata*) wood (Takahashi and Koshijima, 1988a). The uronic acid content would decrease after sodium borohydride reduction if there were any ester-linked LC bonds. The comparison of uronic acid content before and after sodium borohydride treatment indicated that about 30% of the glucuronic acid units in the LCC was in the form of an ester and that the molar ratio of glucuronic acid to xylose was 1:7. Because the glycosidic linkages between sugar units are stable under sodium borohydride

reduction condition, the release of arabinitol and xylitol from the alkaline borohydride treatment of a LCC has lead to the conclusion that xylose and arabinose are directly linked to lignin via phenolic benzyl ether bonds (Takahashi and Koshijima, 1988b).

2.3.4 Smith degradation

Smith degradation is a sequence of reactions including diol cleavages of carbohydrates by sodium periodate, borohydride reduction and mild hydrolysis. Smith degradation has been widely used in structural determination of carbohydrates since sodium periodate will only cleave vicinal diols. This unique property has also made Smith degradation a very useful method for investigating LC linkages in wood (Eriksson and Goring, 1980; Yaku *et al.*, 1981). Periodate oxidation cleaves the bond between C-2 and C-3 of pyranose linking with 1, 4-linkages, but not in the case of the pyranose carrying substituents at C-2 or C-3. Therefore, the sugar units that are linked to lignin in C-2 or C-3 positions should remain intact after LCC is thoroughly oxidized by sodium periodate. Using the Smith degradation method on a neutral LCC, Yaku *et al.* (1981) found that D-mannose linked to both lignin (C-6) and acetyl group (C-2 or C-3), and linked to lignin alone (C-2 or C-3); D-glucose bound lignin only (C-2 or C-3) because D-glucose did not have acetyl group. Using Smith degradation, Eriksson and Goring (1980) demonstrated that lignin was linked to 2- or 3-positions of arabinose units in a LCC isolated from Black spruce (*Picea mariana*).

2.3.5 Methylation method

Methylation is the most commonly used protocol developed for structural analysis of polysaccharides. Methylation of carbohydrates was first performed with dimethyl sulfate and aqueous sodium hydroxide (Purdie and Irvine, 1903; Haworth, 1915). Several methods using methyl iodide in DMF with silver oxide (Kuhn *et al.*, 1955), barium

hydroxide (Kuhn *et al.*, 1958) and sodium hydride (Tamura and Imanari,1964) as the basic reagents were subsequently developed. These methods increase the reaction rates, but full permethylation still can not be achieved. The Hakomori (1964) used methyl iodide, methyl sulphoxide and sodium hydride system to permethylate carbohydrates. The $CH_3SOCH_2^-$, generated from methyl sulphoxide and sodium hydride, is considered as the effective base in the Hakomori method. Hakomori method has been extensively used in structural investigation of carbohydrates. A simple and rapid method for the permethylation of carbohydrates (DMSO-NaOH-methyl iodide system) has also been developed by Ciucanu and Kerek (1984).

Since methylation analysis can provide information on branch points of the sugar residues, the methylation method can be used to determine the linkage points of carbohydrates in LCCs. Indirect evidences for lignin-carbohydrate linkages can be provided after the composition of methylated sugar units in LCCs is compared with that in hemicelluloses. The Hakomori methylation analysis has been used in the investigations of LC linkages (Mukoyoshi *et al.*, 1981, Minor, 1982, 1986 and 1991; Iverson, 1985; Iverson and Wännström, 1986). The use of Ciucanu and Kerek method is also reported recently (Kosíková and Ebringerová, 1994). The above mentioned methods all require a strong base, which implies that methylation analysis is ineffective for the determination of the alkali labile linkages in LCCs. A mild methylation with methyl trifluoromethanesulphonate has been developed (Arnarp *et al.*, 1975 and Arnarp and Lönngren, 1978). Arnarp methylation has been widely used in the analysis of LCCs (Takahashi and Koshijima, 1988b, Watanabe, 1989). It is noteworthy that diazomethane has been used to selectively methylate phenol hydroxyls in the analysis of LCCs (Takahashi and Koshijima, 1988b).

2.4 DDQ method

2.4.1 Introduction

2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) is a valuable oxidative reagent in organic chemistry. Since Becker and Adler (1961) first demonstrated benzyl alcohols could be oxidized to their corresponding α-carbonyl compound by DDQ, numerous applications of DDQ as an oxidant have been reported (Becker *et al.*, 1980; Sadler and Stewardt, 1969; Lutz and Kiefer, 1970; Nakamura and Higuchi, 1976; Oikawa *et al.* 1984a,b; Kim and Misco, 1985). DDQ oxidation in hydrous media proceeds via a charge transfer mechanism in which hydride ion transfers from the benzyl ether to DDQ and subsequent attack by water to the cation intermediate (Oikawa *et al.*, 1984a). Therefore, solvents that can stabilize the charge transfer complex accelerate the oxidation (Ohki *et al.*, 1979) and DDQ oxidation is dependent on the solvents used. Electron-donating groups on the aromatic ring facilitate the oxidation, whereas electron-withdrawing groups retard the oxidation (Becker *et al.*, 1980). Both hydrous and anhydrous solvents have been successfully used in DDQ oxidation. Through the studies of lignin-carbohydrate model compounds, it has been found that DDQ can selectively oxidize benzyl positions such as benzyl alcohols, benzyl ethers and benzyl esters, as well as the γ-position of conjugated arylpropenes (Watanabe, 1989). These unique selectivities of DDQ oxidation have made the detection of LC bonds in LCCs possible. Since Watanabe *et al.* (1987) applied DDQ oxidation to determine LC linkages in the isolated LCCs, DDQ oxidation has become one of the most powerful methods available to investigate lignin-carbohydrate covalent linkages. Various ether and ester linkages in LCCs isolated from different species have been investigated (Watanabe, 1989; Watanabe *et al.*, 1992, 1993; Takahashi and Koshijima, 1988a, b; Imamura *et al.*, 1994).

2.4.2 The mechanism of DDQ oxidation

DDQ can oxidize benzyl alcohol in both hydrous and anhydrous media. However, oxidative cleavage of benzyl ethers and benzyl esters are usually carried out in hydrous media. This can be explained from the mechanism of DDQ oxidation as shown in Figure 1 (Oikawa *et al.*, 1984a).

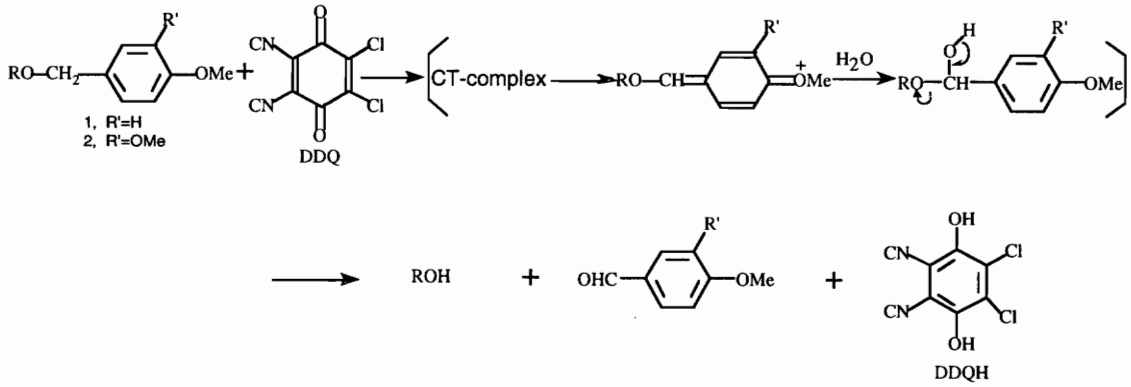


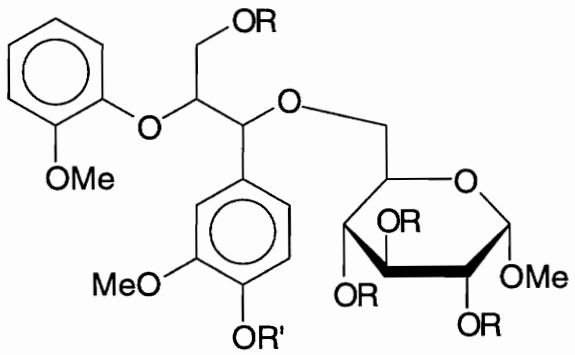
Figure 1. DDQ oxidation of benzyl ethers in the presence of water

Adopted from Oikawa, Y. *et al*, *Tetrahedron Lett.*, 25 (**1984**), 5393

DDQ first forms a charge transfer complex with a substrate. Hydride anion then transfers from the benzyl ether to DDQ, and finally water is added to the resulting benzylcation intermediate. A kinetic study has demonstrated that hydride ion transfer from the α-carbon to DDQ is the rate-determining step (Ohki *et al.*, 1979). From the above mechanism, we can see that solvents that can stabilize the charge transfer complex would accelerate the oxidation. Electron-donating groups on the aromatic ring would stabilize the resulting benzylcation intermediate, and therefore facilitate the oxidation. In contrast, electron-withdrawing substituents on the aromatic ring would destabilize the benzylcation intermediate, retarding the oxidation. Furthermore, a nucleophile is needed to attack the benzylcation intermediate in order to break down the benzyl ether or ester. Similarly, in the oxidation of conjugated arylpropenes, the hydride anion is directly transferred from the γ-position of arylpropenes to DDQ because the resulting carbocation at the γ-position of arylpropenes can be stabilized by conjugation. The resulting cation intermediate is very

reactive to water and the inclusion of water is typical for most DDQ oxidation solvent systems. Dichloromethane-water is the most commonly used solvent system. Because the reduced DDQ product, 2,3-dichloro-5,6-dicyanohydroquinone (DDHQ), has a low solubility in dichloromethane, the oxidation basically proceeds in a neutral media. Therefore, both base-sensitive and acid-sensitive groups such as acetate, isopropylidene, methoxylmethyl, tetrahydropyranyl, tosyl, epoxide and ketone are stable during DDQ oxidation reactions. In addition, the rate of DDQ oxidation depends on the oxidation potential of the benzyl ethers/esters to be cleaved. The lower the oxidation potential, the faster the oxidation. The relative rates of cleavage of several methoxy-substituted benzyl ethers with DDQ have been reported (Nakajima *et al.*, 1988). For example, 3,4-dimethoxybenzyl ether was cleaved with DDQ in presence of 4-methoxybenzyl ether with 98% selectivity (Oikawa *et al.*, 1984b; Matteson and Kandil, 1987). With respect to the application of DDQ oxidation to characterize LC bonds in LCCS, a comprehensive review has been reported (Watanabe, 1989).

2.4.3 DDQ oxidation methods to determine ether linkages in LCCs



I) R=H, R'=H; II) R=Ac, R'=Ac

III) R=Ac, R'=Me; IV) R=Ac, R'=Bn

Figure 2

An ether linkage is one of the most important covalent linkages between lignin and polysaccharides in plants. LC ether linkages can reduce the rate of delignification in pulping processes, and increase the chemical loading required for bleaching. Much attention has been directed to characterize LC ether linkages in wood. As for the use of DDQ in quantifying LC linkages,

selectivities of DDQ oxidation must be tested on appropriate ether-linked LC model compounds before DDQ oxidation can be applied to isolated LCCs. Becker and Adler (1961) first demonstrated that DDQ oxidation of guaiacylglycerol-β-guaiacyl ether provided corresponding α-carbonyl compounds, whereas β- and γ-positions remained unchanged. The following model compounds were synthesized and oxidized by DDQ (Watanabe, 1989) (Figure 2). The oxidative cleavage of the benzyl ether linkages in the LCC model compounds with DDQ is showed in the table below.

Table 1. Oxidative cleavage of the benzyl ether linkages in the LCC model compounds with DDQ[a]

p-Substituents of model compounds			
Hydroxy (**I**)	Acetoxy (**II**)	Methoxy (**III**)	Benzyloxy (**IV**)
100.0	0.0	Trace	50.1

a: Values are expressed as the yield of the corresponding carbonyl compounds (adopted from Watanabe, 1989). See Figure 2 for structures.

The LCC model compound with a free *p*-phenol hydroxyl (**I**) was easily oxidized into the α-carbonyl compound. The yield of α-carbonyl compound from the DDQ oxidation of LCC model compound with a *p*-benzyloxy (**IV**) was moderate, whereas a *p*-acetoxy LCC model compound **II** was stable to DDQ oxidation.

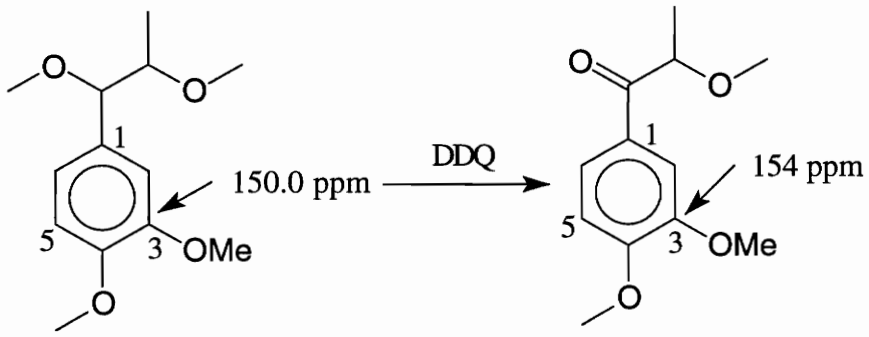


Figure 3

^{13}C NMR was used to characterize the signal changes in untreated and DDQ-treated LCCs (Watanabe and Koshijima, 1988) (Figure 3). The signal at 150.0 ppm was proposed to originate from C-3 in etherified guaiacyl nuclei accompanied by benzyl ether linkages (Nimz *et al.*, 1981); the signal at around 154 ppm was assigned C-3 in *p*-etherified guaiacyl nuclei with α-carbonyl group (Lüdemann and Nimz, 1974). The disappearance of the signal at 150.0 ppm and the concomitant enhancement of the signal at around 154 ppm were used to suggest that a benzyl ether was oxidized into the α-carbonyl group by DDQ (Watanabe and Koshijima, 1988).

A sequence to prove a LC ether bond in LCCs is described below. A water-soluble LCC was thoroughly digested with a commercial cellulase in order to enhance the frequency of LC linkages. The cellulase-treated LCC was then acetylated with acetic anhydride in pyridine, and the acetylated LCC fragments were oxidized with DDQ in dichloromethane-water. The products from chloroform extraction of the DDQ oxidation mixture were methylated with methyl trifluoromethanesulfonate and 2,6-di-(*tert*-butyl)pyridine in trimethyl phosphate (Prehm, 1980) and then hydrolyzed with 2M trifluoroacetic acid. The hydrolyzate was reduced with sodium borohydride to provide alditols which were subsequently acetylated, and finally separated with gas-liquid chromatography (GLC). The component sugars corresponding to each peaks in GLC were identified by gas chromatography-mass spectroscopy (GC-MS). It has been demonstrated that glycosidic bonds in the acetates of lactose, maltose, gentiobiose, raffinose and cellobiose were stable to DDQ oxidation (Watanabe and Koshijima, 1989; Watanabe *et al.*, 1989). Therefore, the methylated points in each sugar components are the binding sites of carbohydrates to benzyl position of lignin or γ-position of conjugated arylpropene units in lignin. It is noteworthy that DDQ oxidation depends heavily on the substituents on the aromatic ring. DDQ oxidation was only tested on a limited number of LCC model compounds. Additional LCC model compound studies on DDQ oxidation have revealed that the yields

are generally low (Sipilä *et al.*, 1996). Therefore, care must be taken when interpreting results, especially the amount of ether linkages from DDQ oxidation studies. It would be more appropriate with this method to characterize ether linkages qualitatively rather than quantitatively.

2.4.4 DDQ oxidation methods to determine ester linkages in LCCs

Kim and Misco (1985) first demonstrated that 2,4-dimethoxylbenzyl and 2,4,6-trimethoxybenzyl esters could be oxidized by DDQ to provide corresponding benzaldehydes. The oxidation mechanism was proposed to be the same as DDQ oxidation of benzyl ethers, *i.e.* formation of an initial charge transfer complex, hydride anion transfer from benzyl ester to DDQ and subsequent attack of water on the resulting cation intermediate. Watanabe and Koshijima (1988a) applied DDQ oxidation to a benzyl glucuronate and methyl glucuronate, and found that benzyl glucuronate could be oxidized in benzene-water to provide benzaldehyde in 40% yield, whereas methyl glucuronate was stable to DDQ oxidation. Watanabe and Koshijima (1988a) then used this DDQ oxidation to determine LC ester linkages in an LCC isolated from pine wood (*Pinus densiflora*). The acidic subfraction of a water-soluble LCC was treated with diazomethane to block phenol hydroxyls and free uronic acid moieties. The methylated LCC was oxidized by DDQ in aqueous benzene; the resulting uronic acid was separated with anion-exchange chromatography, and then subjected to structural analysis by NMR and titrimetry.

As mentioned earlier, DDQ oxidation of benzyl ethers in aqueous benzene or dichloromethane provided benzaldehyde in low yield. In order to better characterize LC ester linkages in plants, an improved procedure has been reported (Imamura *et al.*, 1994). DDQ oxidation was conducted in the presence of a protonic acid such as trifluoroacetic acid (TFA) in this improved procedure. Protonic acids are known to enhance the

electrophilicity of DDQ (Beresford *et al.*,1974; Lee and Harvey, 1988; Pelter *et al.*, 1991; Handoo and Gadru, 1986). Beresford *et al.* (1974) proposed that the active species for the DDQ oxidation is a conjugate acid between DDQ and protonic acid, which is more electrophilic than DDQ. DDQ oxidation in presence of a protonic acid is believed to follow the same hydride transfer mechanism as that in neutral aqueous systems (Lee and Harvey, 1988; Pelter *et al.*, 1991). On the other hand, a one-electron transfer mechanism was proposed in the oxidation of electron-rich aromatic and heterocyclic molecules by DDQ in dichloromethane-trifluoroacetic acid (Handoo and Gadru, 1986). In this mechanism, the active species is the quinone itself instead of protonated DDQ; one electron transfer produces a cation radical and a semiquinone radical. DDQ oxidation in the presence of a protonic acid (DDQ/TFA system) can be used to characterize LC ester linkages for the following reasons. When DDQ oxidation is used in the analysis of benzyl ether bonds between lignin and carbohydrates, stability of glycosidic bonds during the DDQ oxidation is required to discriminate the benzyl ether bonds from the glycosidic bonds between sugar units. However, cleavage of the glycosidic bonds during DDQ oxidation is no longer an issue in the binding site analysis of LC ester linkages, because the LC ester linkage is determined by detection of the carboxyl group of the glucuronic acid residues. Benzyl glucuronates in several LCC model compounds were quantitatively cleaved in the DDQ/TFA system (Imamura *et al.*, 1994), and the system has been applied to characterizing LC ester linkages in LCCs isolated from beech wood(*Fagus crenata*). In this investigation, an acidic subfraction of a water soluble LCC was ethylated with diazoethane and acetylated with acetic anhydride in pyridine. The resulting sample was oxidized by DDQ/TFA in dichloromethane. The DDQ/TFA treated LCC was neutralized with sodium hydroxide and titrated with perchloric acid to determine the amount of free glucuronic acid formed from the oxidation. Thus the frequency of the ester bonds between lignin and glucuronic acid residue of glucuronoxylan was determined to 1.6 per molecule of the water soluble LCC. It is noteworthy that migration of 4-*O*-methyl glucuronosyl

group between α and γ positions of arylpropane side chain has been observed in neutral and acidic conditions (Li and Helm, 1995). Moreover, LCC model compounds used to test DDQ/TFA oxidation are simple; discrimination of the free glucuronic acid from the other acids such as cinnamic acid was not investigated. Therefore, care must be taken in interpreting the DDQ oxidation results about frequency of ester linkages between lignin and glucuronic acids.

2.5 Model compound methods

The plant cell wall is a complicated 3-dimensional biopolymer composite. The detailed structural information, especially about component interactions, cannot be directly obtained from an intact cell wall system. The investigations in component interactions rely heavily on model systems and cell wall isolates. Information gathered from cell wall isolates can help to establish a better model system; data acquired from a model system can help to develop new analytical methods for the determination of component interactions, as well as help to investigate biosynthetic pathways in the cell wall system. Model compounds can be prepared to represent a variety of structural types in lignin and possible lignin-carbohydrate interactions. Model compound methods play very important roles in many areas of wood chemistry. For example, lignin and lignin-carbohydrate model compounds are widely used in the biodegradation of wood components because the reactivity data that would be difficult to extract from the reaction on polymer are easily obtained from appropriate model compounds (Eriksson *et al.*, 1990; Higuchi, 1990). In a model compound study, Enoki *et al.* (1983) found that phenolic benzyl ether bonds were easily hydrolyzed in an alkaline solution, whereas non-phenolic benzyl ether bonds were stable to alkaline hydrolysis. Borohydride reduction and DDQ oxidation methods were tested on appropriate model compounds before they were used in the investigations of LC linkages (Takahashi and Koshijima, 1988a, Watanabe,1989). Migration of 4-*O*-methyl-

glucuronosyl group between the α- and γ-positions of an arylpropane sidechain was observed through the model compound study (Li and Helm, 1995). Mechanism of formation of non-cyclic benzyl ethers during lignin biosynthesis has been studied in detail by using model compounds (Sipilä and Brunow, 1991a,b,c; Brunow *et al.* 1989). Ralph *et al.* (1992) demonstrated incorporation of methyl 5-*O*-(*E*)-feruloyl-α-L-arabinofuranoside into a synthetic lignin dehydrogenation polymer (DHP) of coniferyl alcohol. This implies that feruloyl esters are also incorporated into the lignin structure through copolymerization with lignin monomers. This also implies that there might exist another mechanism other than quinone methide addition by which feruloyl esters become bound to lignin, and carbohydrates are covalently linked to lignin via feruloyl esters. High-resolution NMR data of structures of interests can be obtained from model compounds. Ralph (1988) demonstrated that ^{13}C NMR chemical shifts in appropriate low molecular weight compounds were identical to those in the polymer. Applying NMR experiments to synthetic α- and γ-*p*-coumaroylated lignin model compounds and isolated maize lignin, Ralph *et al.* (1994) found that *p-coumaric* acid was attached exclusively at the γ-position of phenylpropane side chain in maize lignin. Several lignin minor structures such as diarylpropane-1,3-diols (**I**), α, β-diaryl ether structure(**II**) and arylglycerol (**III**) (Figure 4) have been directly detected in milled wood lignin(MWL) isolated from softwood by the application of 2D and 3D NMR techniques to lignin model compounds and MWLs (Kilpeläinen *et al.*, 1994a,b). A novel type of linkage, dibenzodioxocin (**IV**), has been found in softwood lignin after the comparison of 2D NMR data from a model compound and from isolated softwood lignin (Karhunen *et al.*, 1995a,b).

Direct detection of lignin-carbohydrate linkages in an isolated LCC by NMR techniques has not been reported so far. One of the major reasons for this lack is that the NMR database derived from LCC model compounds is not adequate.

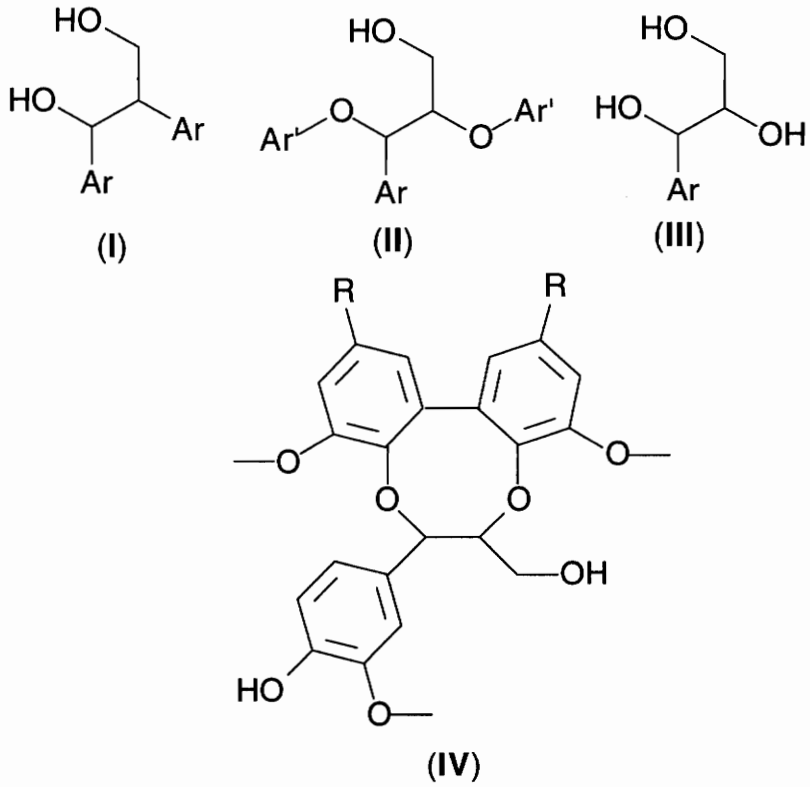


Figure 4. Several lignin minor structures

2.6 Referenses

Arnarp, J. and Lönngren, J., *Acta. Chem. Scand.* B 32 (**1978**) 465.

Arnarp, J.; Kenne, L.; Lindberg, B. and Lönngren, J., *Carbohydr. Res.,* 44 (**1975**) c5.

Azuma, J-I.; Takahashi, N. and Koshikima, T., *Carbohydr. Res.*, 93 (**1981**) 91.

Becker, H. D. and Adler, E., *Acta Chem. Scand.*, 15 (**1961**) 218.

Becker, H. D.; Björk, A. and Adler, E., *J. Org. Chem.*, 45 (**1980**) 1596.

Björkman, A., *Svensk Papperstidn.,* 59 (**1965**) 477.

Björkman, A., *Ind. Eng. Chem.*, 47 (**1957**) 1395.

Björkman, A. and Person, B., *Svensk, Papperstidn.*, 60 (**1957**) 158.

Brauns, F. E., *J. Am. Chem. Soc.*, 61 (**1939**) 2120.

Brauns, F. E., *The Chemistry of lignin*, Academic Press, New York, (**1952**) 49-132, 742-744.

Beresford, P.; Ales, D.H.; Krika, L.J. and Ledwith, A., *J. Chem. Soc. Perkin Trans. 1,* (**1974**) 276.

Brunow, G; Sipilä, J. and Makela, T., *Holzforchung*, 43 (**1989**) 55.

Ciucanu, I. and Kerek, F., *Carbohydr. Res.,* 131 (**1984**) 207.

Comtat, J.; Joseleau, J.-P.; Bosso, C.and Barnoud, F., *Carbohydr. Res.* 38 (**1974**) 217.

Enoki, A.; Yaku, F. and Koshijima, T., *Holzforschung*, 37 (**1983**) 135-141.

Eriksson, K.-E. L.; Blanchette, R. A. and Ander, P., *Microbial and enzymatic degradation of wood and wood components*; Springer-Verlag, New York, Berlin, Heidelberg, **1990**, 225-333.

Eriksson, O. and Lindgren, B. O., *Svensk Papperstidn.*, 2 (**1977**) 59.

Eriksson, O and Goring, D. A. I, *Wood Sci. Technol.*, 14 (**1980**) 267.

Ford, C. W. and Elliott, R., *J. Agric. Sci.*, 108 (**1987**) 201.

Hakomori, S., *J. Biochem. (Tokyo)*, 55 (**1964**) 205.

Handoo, K. L. and Gadru, K. *Curr. Sci.,* 55 (**1986**) 920.

Haworth, W. N., *J. Chem. Soc.*, 107 (**1915**) 8.

Higuchi, T., *Wood Sci. Technol.*, 24 (**1990**) 23.

Imamura, T.; Watanabe, T.; Kuwahara, M. and Koshijima, T., *Phytochemistry* , 37 (**1994**) 1165.

Iversen, T., *Wood Sci. Technol.*, 19 (**1985**) 243.

Iversen, T. and Wännström S., *Holzforschung*, 40 (**1986**) 19.

Jansson P.-E.; Keene, L.; Liedgren, H.; Lindberg, B. and Löunngren, J., *Chem. Commun., Univ. of Stockholm* , No. 8 (**1976**).

Jeffries, T. W., *Biodegradation of lignin and hemicelluloses.* in *Biochemistry of Microbial Degradation* ., Ratledge, C., Ed., Kluwer Academic Publishers,

Dordrecht Boston London., **1994**, pp 233-277.

Johnson, K. G. and Overrend, R. P., *Holzforchung*, 46 (**1992**) 31.

Joseleau, J.-P. and Kesraoui, R., *Holzforschung*, 40 (**1986**) 163.

Karhunen, P.; Rummakko, P.; Sipilä, J.; Brunow, G. and Kilpeläinen, I., *Tetrahedron Lett.,* 36 (**1995**) 4501.

Karhunen, P.; Rummakko, P.; Sipilä, J.; Brunow, G. and Kilpeläinen, I., *Tetrahedron Lett.,* 36 (**1995**) 169.

Katayama, Y.; Morohoshi, N. and Haragushi, T., *Mokuzai Gakkaishi*, 26 (**1980**) 414.

Kilpeläinen, I; Sipilä, J.; Brunow, G.; Lundquist, K. and Ede, R.M., *J. Agric. Food Chem.,* 42 (**1994**) 2790.

Kilpeläinen, I; Ammalahti, E.; Brunow, G. and Robert, D., *Tetrahedron Lett.*, 35 (**1994**) 9267.

Kim, C. U. and Misco, P. F., *Tetrahedron. Lett.*, 26 (**1985**) 2027.

Kosíková, B. and Ebringerová, A., *Wood Sci. Technol.*, 28 (**1994**) 291.

Kosíková, B.; Joniak, D. and Kosáková, L., *Holzforschung*, 33 (**1979**) 11.

Kuhn, R.; Trischmann, H. and Low, I., *Angew. Chem.*, 67 (**1955**) 32.

Kuhn, R.; Baer, H. H. and Seeliger, A., *Justus Liebigs Ann.Chem.*, 611 (**1958**) 236.

Lai, Y. Z. and Sarkanen, K. V., *Isolation and structural studies.* in *Lignin, Occurrence, Formation, Structure and Reactions.*, Sarkanen, K. V.; Ludwig, C. H.; Eds.; Wiley-Interscience, New York, **1971**, pp165-240.

Lee, H. and Harvey, R. G., *J. Org. Chem.* 53 (**1988**) 4587.

Li, K. and Helm, R.F., *J. Agric. Food Chem.* 43 (**1995**) 2098.

Lüdemann H.-D. and Nimz, H. H., *Macromol. Chem.*, 175 (**1974**) 2393.

Lundquist, K.; Simonson, R. and Tingsuik, K., *Svensk Papperstidn.*, 86 (**1983**) 44.

Lutz, F. E. and Kiefer, E. F., *Tetrahedron Lett.*, 11 (**1970**) 4851.

Matteson, D. S. and Kandil, A. A., *J. Org. Chem.,* 52 (**1987**) 5121.

Merewether, J. W. T. and Samsuzzaman, L. A. M., *Holzforschung*, 26 (**1972**) 11.

Minor, J. L., *J. Wood Chem. Technol.*, 11 (**1991**) 159.

Minor, J. L., *J. Wood Chem. Technol.*, 6 (**1986**) 185.

Minor, J. L., *J. Wood Chem. Technol.*, 2 (**1982**) 1.

Mukoyoshi, S. I.; Azuma, J. I. and Koshijima, T., *Holzferschung,* 35 (**1981**) 233.

Nakajima, N.; Abe, R. and Yonemitsu, O., *Chem. Pharm. Bull.*, 36 (**1988**) 4244.

Nakamura, Y. and Higuchi, T., *Wood Res.*, (**1976**) 101.

Neilson, M. J. and Richards, G. N., *Carbohydr. Res.* 104 (**1982**) 121.

Nimz, H. H.; Robert, D.; Faix, O. and Xemr, M., *Holzforshung*, 35 (**1981**) 16.

Obst, J. R., *Tappi*, 65 (**1982**) 109.

Ohara, S.; Hosoya, S. and Nakano, J., *Mokuzai Gakkaishi*, 26 (**1980**) 408.

Ohki, A.; Nishiguchi, T. and Fukuzimi, K., *Tetrahedron* , 35 (**1979**) 1737.

Oikawa, Y.; Tanaka, T.; Horita, K. and Yonemitsu, O., *Tetrahedron Lett.,* 25 (**1984**) 5397.

Oikawa, Y.; Tanaka, T.; Horita, K.; Yoshioka, T. and Yonemitsu, O., *Tetrahedron Lett.*, 25 (**1984a**) 5393.

Pew, J. C., *Tappi*, 40 (**1957**) 553.

Pelter, A.; Ward, R. S.; Venkateswarlu, R. and Kamakshi, C., *Tetrahedron* , 47 (**1991**) 1275.

Prehm, P. *Carbohydr. Res.*, 78 (**1980**) 372.

Purdie, T. and Irvine, J. C., *J. Chem. Soc.,* 83 (**1903**) 1021.

Ralph, J., *Holzforshung*, 42 (**1988**) 273.

Ralph, J.; Hatfield, R. D.; Quideau, S; Helm, R. F.; Grabber, J. H. and Jung, H-J. G., *J. Am. Chem. Soc.,* 116 (**1994**) 9445.

Ralph, J.; Helm, R. F.; Quideau, S. and Hatfield, R. D., *J. Chem. Soc. Perkin Trans. 1*, (**1992**) 2961.

Sadler, I. H. and Stewart, J. A. G., *J. Chem. Soc. (c)* , (**1969**), 773.

Sipilä, J. and Brunow, G., *Holzforschung,* 45 (**1991**) 275.

Sipilä, J. and Brunow, G., *Holzforschung,* 45 (**1991**) Suppl. 3.

Sipilä, J. and Brunow, G., *Holzforschung*, 45 (**1991**) Suppl, 9.

Sipilä, J.; Brunow, G.; Leikoski, T. and Vuorensola, K., *Oxidation of LCC benzyl ether model compounds with DDQ.* 211th ACS National ACS meeting, New Orleans, March 24-28, **1996**, CELL-053.

Smelstorius, J. A., *Holzforschung*, 28 (**1974**) 99.

Staudinger, H.; Dreher, E. and Ekenstam, A., *Chem. Ber.*, 69B (**1936**) 1099.

Takahashi, N. and Koshijima, T., *Wood Sci. Technol.*, 22 (**1988a**) 231.

Takahashi, N. and Koshijima, T., *Wood Sci. Technol.*, 22 (**1988b**) 177.

Tamura, Z. and Imanari, T., *Chem. Pharm Bull.*, 12 (**1964**) 1386.

Tanaka, K.; Nakatsubo, F. and Higuchi, T., *Mokuzai Gakkaishi*, 25 (**1979**) 653.

Wang, P.Y.; Bolker, H.I. and Purves, C. B., *Tappi*, 50 (**1967**) 123.

Watanabe, T., *Wood Research* , 76 (**1989**) 59.

Watanabe T. and Koshijima, T., *Agric. Biol. Chem.*, 52 (**1988b**) 2953.

Watanabe, T. and Koshijima, T., *Wood Res.*, (**1988b**) 13.

Watanabe, T. and Koshijima, T., *Mokuzai Gakkaishi*, 35 (**1989**) 130.

Watanabe, T. ; Ohnishi, J.; Yamasaki, Y.; Kaizu, S. and Koshijima, T., *Agric. Biol. Chem.*, 53 (**1989**) 2233.

Watanabe, T.; Azuma, J. and Koshijima T., *Mokuzai Gakkaishi*, 33 (**1987**) 798.

Watanabe, T.; Karina, M.; Sudiyani, Y.; Koshijima, T. and Kuwahara, M., *Wood Res.*, 79 (**1993**) 13.

Yaku, F.; Tanaka, R. and Koshijima, T., *Holzforschung*, 35 (**1981**) 177.

Yaku, F.; Yamda, Y. and Koshijima, T., *Holzforschung*, 30 (**1976**) 148.

Chapter 3

Approaches to the Synthesis of Woody Plant Cell Wall Model Compounds

3.1 Synthesis of Lignin Model Compounds

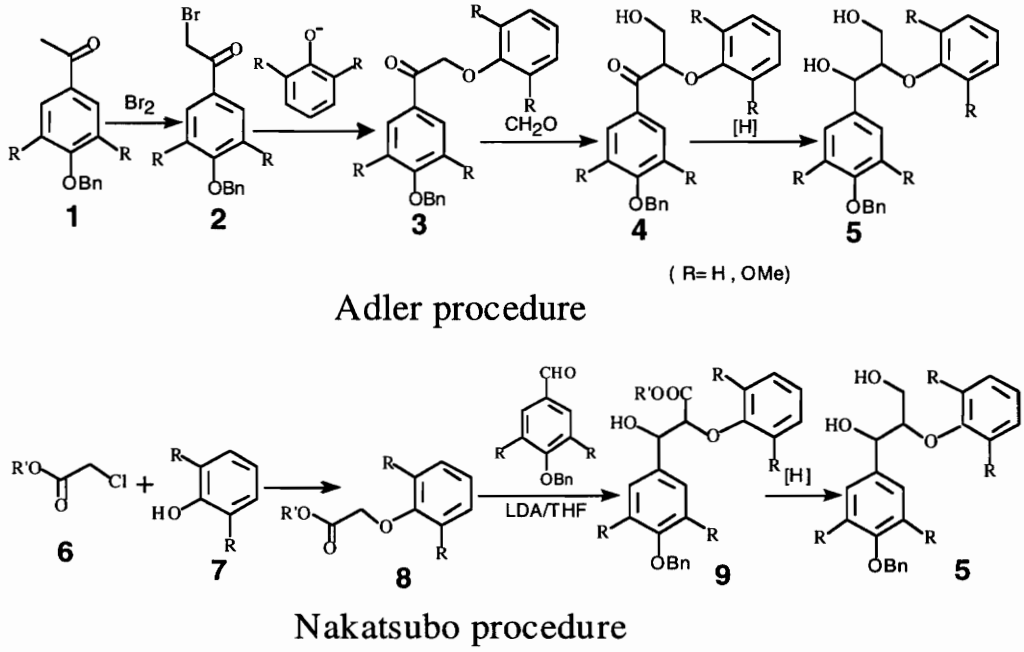
3.1.1 Introduction

Lignin is one of the most complex natural polymers. It is highly branched and lacks regular and ordered repeating units such as in the case of cellulose or proteins. Lignin structure varies with different species, as well as within different regions of an individual tree. As described in Chapter 1, free radical coupling in lignin biosynthesis provides various types of interunit linkages such as β-*O*-4, β-β, β-*O*-5, 5-5, 4-*O*-5, *etc.* Various lignin model compounds from dimers to hexamers have been synthesized in order to investigate the reactivity of lignin. Methods to prepare lignin model compounds are reviewed below. Oxidative coupling of arylpropene derivatives by using one-electron oxidants such as ferric chloride, peroxidases-H_2O_2 and so on provided several dimeric lignin model compounds and polymeric materials in one pot (Landucci, 1995; Leopold, 1950; Sarkanen Wallis, 1973; Wallis, 1973; Katayama and Fukuzumi, 1978). This oxidative coupling method once provided convenient access to lignin model dimers, but it was usually less efficient to prepare the desired lignin model compounds. This oxidative coupling method to prepare lignin model compounds will not be reviewed here.

3.1.2 General approaches

3.1.2.1 The synthesis of β-*O*-4 lignin dimers

Arylglycerol-β-aryl ether (β-*O*-4) is the most abundant interunit linkage, and accounts for 40-60% of interunit linkages in lignin (Adler, 1977). As a result, β-*O*-4 lignin dimers are very useful in the investigation of lignin structure and reactivity. Several synthetic routes have been developed to prepare β-*O*-4 lignin model dimers, although two methods find common use (Scheme 1). The one procedure was developed by Adler (Adler *et al.*, 1952; Adler and Eriksoo, 1955); the other was developed by Nakatsubo (Nakatsubo *et al.*, 1975). The Adler procedure constructs an arylpropane sidechain in a linear fashion starting with an acetophenone derivative, whereas the Nakatsubo procedure uses a convergent strategy. The reaction conditions in the Adler procedure are more convenient than those in Nakatsubo procedure in which aldol type reaction step is air and moisture sensitive. However, the Nakatsubo procedure is one step shorter than Adler procedure.

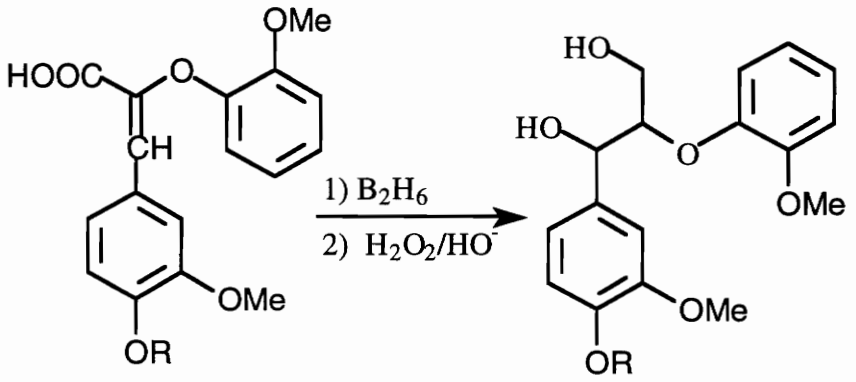


Scheme 1

In the Adler procedure, diastereoselectivity of the final products depends on reagents and

solvents used in the reduction of the α-ketone **4**. Depending on the reductant and solvents used, different ratios of *threo* and *erythro* isomers have been observed (Brunow *et al.*, 1988; Ralph and Helm, 1991; Ralph and Young, 1981). Sodium borohydride reduction of the α-ketone **4** derivatives is usually used to provide the *threo* enriched mixture of *threo* and *erythro* isomers. A complete *threo* stereospecific preparation of β-*O*-4 lignin model dimer has recently been developed by the reduction of γ-hydroxyl-protected ketone **4** with DIP-Cl (cf. Chapter 4, Helm and Li, 1995). High *erythro* stereoselectivity (up to 97%) can be achieved by the reduction of the γ-hydroxyl-protected α-ketone **4** with zinc borohydride (Helm and Ralph, 1993).

In the Nakatsubo procedure, an aldol type condensation step determines the overall stereochemistry of the final β-*O*-4 model dimers. It has been showed that condensation of ethyl or methyl esters (R'=Et or Me in **8**) provides mainly *erythro* isomers, whereas a bulky alcohol component in the ester **8** (R' is a bulky group) alters the stereoselectivity in the condensation, and *threo* isomers are predominantly formed. The best selectivities are 70-90% *threo* isomers (Brunow *et al.*, 1988). In the less frequently used methods to prepare lignin model dimers, hydroboration-oxidation of α-(2-methoxyphenoxy)-3,4-dimethoxycinnamic acid has been investigated (Scheme 2) (Ahvonen *et al*, 1983; Lundquist and Remmerth, 1975; Berndtsson and Lundquist, 1977; Brunow and Lundquist, 1984a;



10 R=Bn
11 R=Me
12 R=Bn
13 R=Me

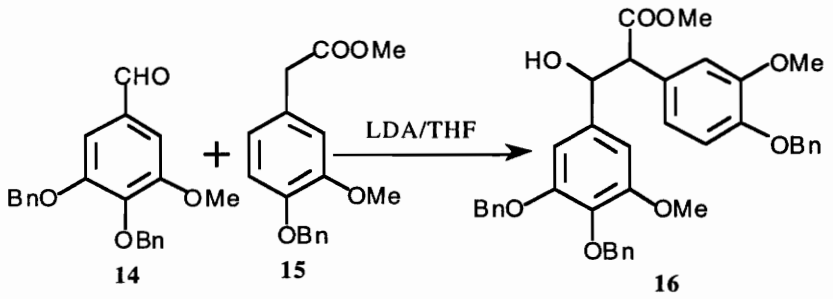
Scheme 2

Brunow *et al.*, 1988). *Erythro* isomers of compounds **12** and **13** were prepared from hydroboration-oxidation of the Z-forms of compounds **10** and **11**, respectively, in a yield of about 50%, whereas the same hydroboration-oxidation of E- forms of compounds **10** and **11** provided *threo* isomers of compounds **12** and **13** together with minor amount of *erythro* isomers and large amounts of byproducts. Another less frequently used method was developed by Pearl and Gratzl (1962). Synthesis of a series of β-*O*-4 lignin model dimers having different substituents on the aromatic rings has been reported (Sipilä and Syrjanen, 1995). It should be noted that a new method to separate *threo* and *erythro* isomers of β-*O*-4 lignin model dimers has recently been developed by use of anion exchange chromatography (Ibrahim and Lundquist, 1994). A rapid proton NMR method for determining *threo*:*erythro* ratios of β-*O*-4 model dimers has also been established (Ralph and Helm, 1991).

3.1.2.2. Synthesis of lignin model dimers other than β-*O*-4 types

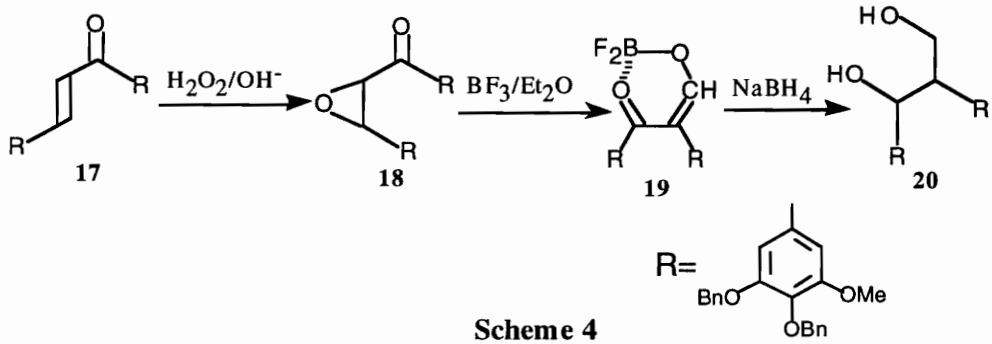
Besides the β-*O*-4 linkage, there are many other important interunit linkages in lignin (cf. Chapter 1). Since they are found in lesser amounts, correspondingly less synthetic work has been done. Several synthetic methods have been used to prepared β-1 lignin dimers (Lundquist and Miksche, 1965; Nakatsubo and Higuchi, 1975; Brezny and Pufflerova, 1978; Kristersson and Lundquist, 1980; Berndtsson *et al.*, 1980; Nonni and Dence, 1982; Ahvonen *et al.*, 1983).

Two of these methods are representative. Nakatsubo and Higuchi (1975) synthesized β-1 lignin dimers (1,2-diarylpropane type

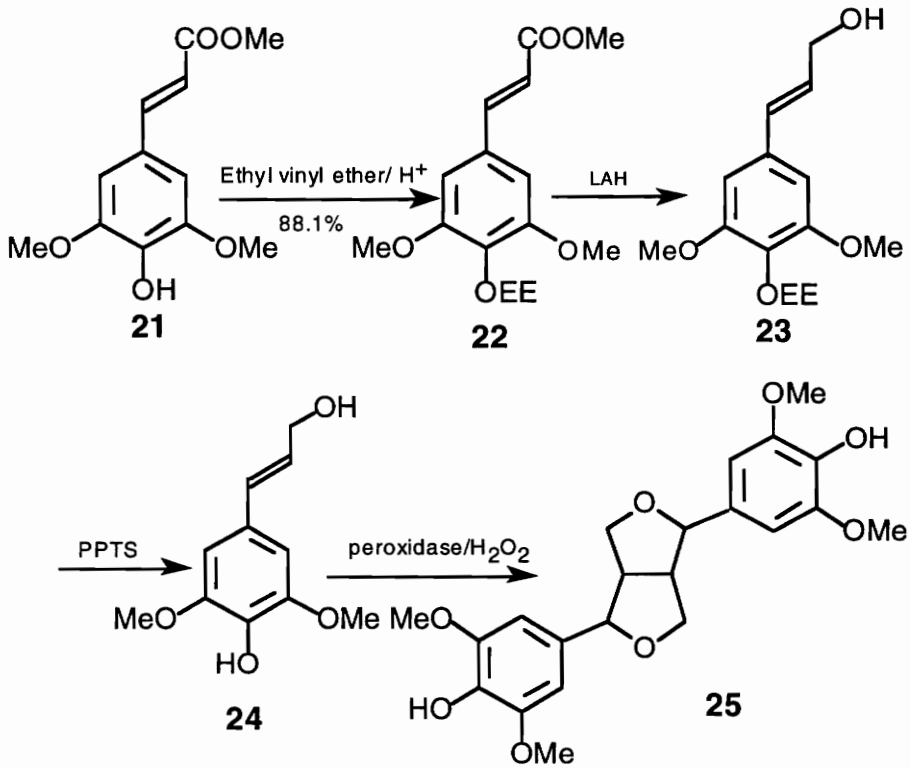


Scheme 3

linkage) by the reaction of methyl 2-arylacetate, lithium diisopropyl amide (LDA) with a benzaldehyde derivative (Scheme 3). Another route is through the rearrangement of an α,β-epoxy ketone (House and Ryerson, 1961; Lundquist and Miksche, 1965; Berndtsson *et al.*, 1980; Kristersson and Lundquist, 1980; Ahvonen *et al.*, 1983) (Scheme 4). Sodium borohydride reduction of **19** is stereoselective providing *erythro* isomers of **20** in high yield.



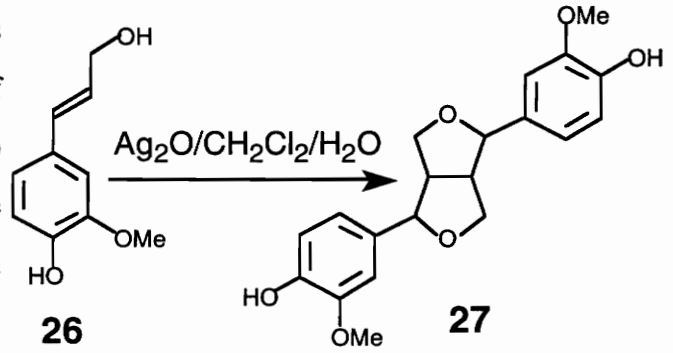
Scheme 4



Scheme 5

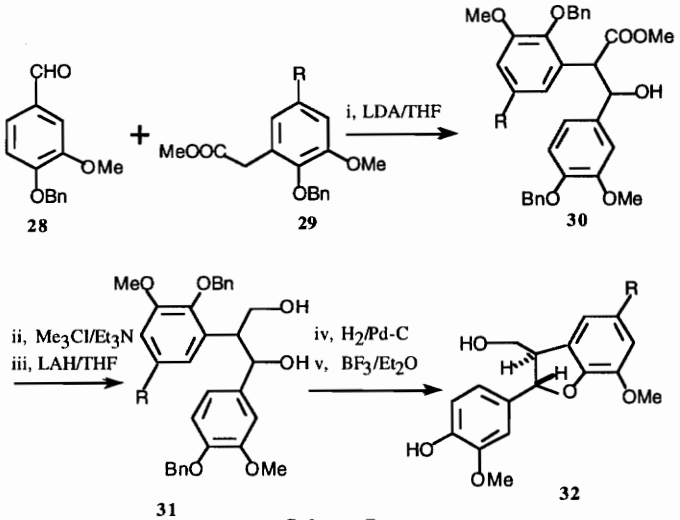
An excellent synthetic route has been developed to prepare a representative lignin model dimer of the β-β interunit linkage, syringaresinol **25** (Scheme 5) (Kamaya *et al.*, 1980). The synthesis started from readily available methyl sinapate **21**. The key step is the oxidation of sinapyl alcohol **24** with peroxidase-hydrogen peroxide. The overall yield is around 59% based on methyl sinapate **21**.

About 25% of pinoresinol (**27**) was isolated from the oxidation of coniferyl alcohol with silver (I) oxide in 1:1 methylene chloride:water (Scheme 6) (Quideau and Ralph, 1994).



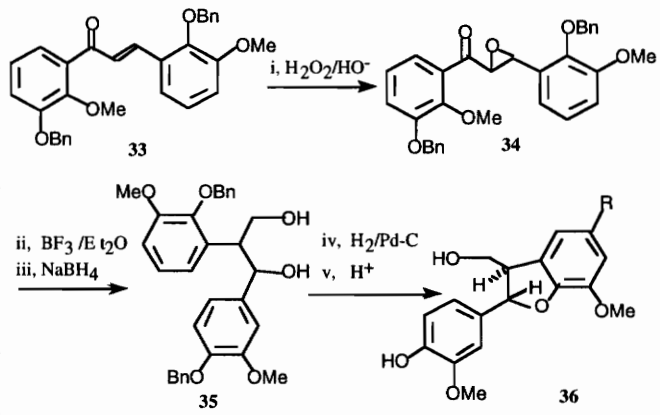
Scheme 6

The phenylcoumaran interunit linkage (β-5) is one of the more important coupling modes in lignin. The reactivity of phenylcoumaran structure will be useful in investigations of lignin reactions such as pulping, chemical utilization and biodegradation of lignin. Moreover, the isolation of some optically active phenylcoumarans from plant extractives has also promoted interests in the investigation of biosynthetic difference between optically inactive lignin and optically active lignans (Ichihara *et al.*, 1976, 1977, 1978; Popoff and Theander, 1975, 1977; Takemoto *et al.*, 1975). Several



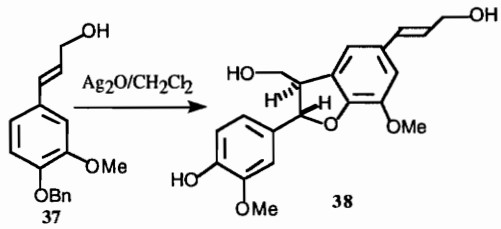
Scheme 7

synthetic methods have been developed to prepare phenylcoumarans (Nakatsubo and Higuchi, 1979; Brunow and Lundquist, 1984b; Quideau and Ralph, 1994). Nakatsubo and Higuchi (1979) first developed a synthetic route for preparation of phenylcoumaran (Scheme 7). Only *threo* isomers of **30** were formed in the Aldol condensation of **28** and **29**. Direct hydride reduction of **30** under various conditions provided **31** in low yield (an α-deoxy derivative of the diol **31** was reported to be the main product under these reductions). Protection of the hydroxyl group in **28** as a silyl ether and subsequent LAH reduction provided the diol **31** in 85% yield. Debenzylation of **31** and subsequent ring closure in presence of a catalytic amount of BF_3 successfully provided the *trans* phenylcoumaran **32** in 85% yield. Brunow and Lundquist (1984b) developed another method to synthesize phenylcoumaran as shown in Scheme 8. Key intermediate **35** was synthesized by rearrangement of α,β-epoxy ketone **34** and subsequent sodium borohydride reduction. The cyclization in **35** was easily accomplished in the presence of a catalytic amount of hydrochloric acid, providing **36**.



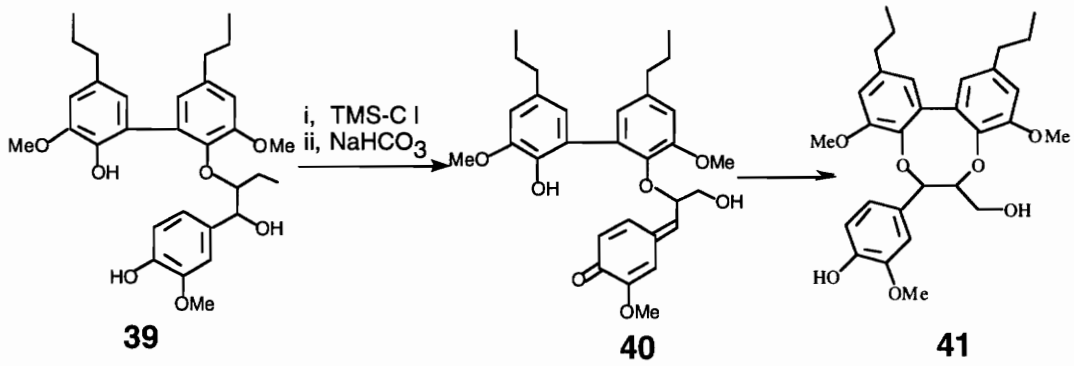
Scheme 8

Quideau and Ralph (1994) recently developed a convenient method to prepare phenylcoumaran **38** (Scheme 9). (*E*)-coniferyl alcohol **37** was oxidized by silver (I) oxide in dry dichloromethane to provide **38** in about 50% yield.



Scheme 9

Karhunen *et al.* (1995a) synthesized a dibenzodioxocin **41** that represents a novel type of linkage in lignin (Scheme 10). The β-aryl ether **39** was synthesized following the Adler procedure (Adler *et al.*, 1952). The reaction of **39** with trimethylsilyl bromide and subsequent aqueous sodium bicarbonate provided the quinone methide **40**. Intramolecular nucleophilic addition to quinone methide **40** provided a dibenzodioxocin **41**. This novel type of linkage has been found in softwood lignin by NMR using the model compounds as a database (Karhunen *et al.*, 1995b).



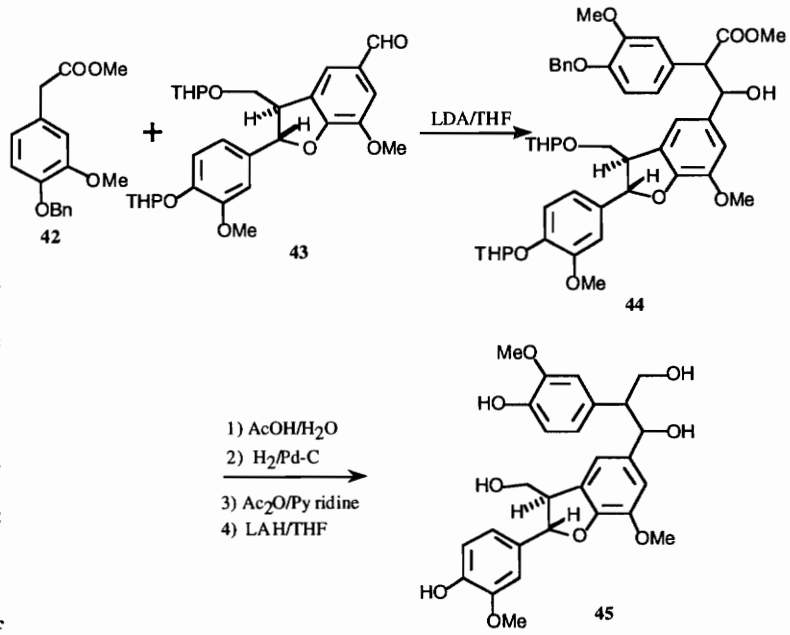
Scheme 10

3.1.2.3 Synthesis of oligomeric lignin model compounds

Efforts towards the synthesis of oligomeric lignin model compounds are based on the need for more accurate modeling of lignin for structural and reactivity studies. Oligomeric lignin model compounds that contain different types of interunit linkages are very useful for studying the reactions of lignin during pulping, bleaching and/or biodegradation. By means of NMR data from oligomeric lignin model compounds, it has been demonstrated that chemical shifts of sidechain and free-phenolic end of lignin dimers are in good agreement with higher oligomers and lignin polymers itself (Ralph, 1988). Moreover, oligomeric lignin model compounds, especially trimers, can provide valuable NMR data which can be used to accurately determine both the free-phenolic and the internal units in

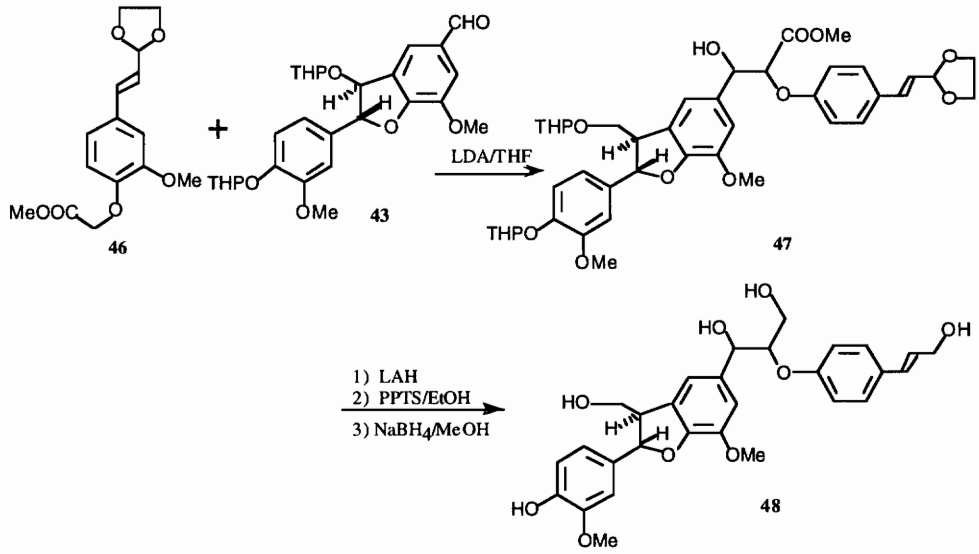
lignin. An optically inactive trimer having phenylcoumaran and a β-1 structures has been isolated from the lignin hydrolysis products of Ezomatsu (*Picea jezensis*), whereas the same trimer isolated from the heartwood extractives of Japanese larch (*Larix leptolepis*) is optically active (Miki, *et al.*, 1979).

A series of trimeric lignin model compounds have been synthesized. Nakatsubo and Higuchi (1980a, b) prepared two trimeric lignin model compounds composed of phenylcoumaran/β-1 and phenylcoumaran/β-*O*-4 interunit linkages (Schemes 11 and 12). Compound **44** was prepared in 87% yield by the aldol type condensation of an ester **42** and a benzaldehyde derivative having a phenylcoumaran structure **43** (Scheme 11). The synthesis of the benzaldehyde derivative **43** is based on the procedure described in Scheme 7. Conversion of **44** to phenylcoumaran/β-1 trimer **45** can be accomplished in two ways: 1) LAH reduction of the ester **44** and subsequent deprotection; 2) removal of the protecting groups, peracetylation, and finally LAH reduction. It was found that LAH reduction of **44** caused severe retro-aldol condensation, and only about 40% of the expected diol was obtained. Therefore, the second route was deemed more appropriate. Compound **45** was prepared from **44** in 71% overall yield by acidic deprotection of THP, debenzylation, acetylation of the resulting hydroxyls, and

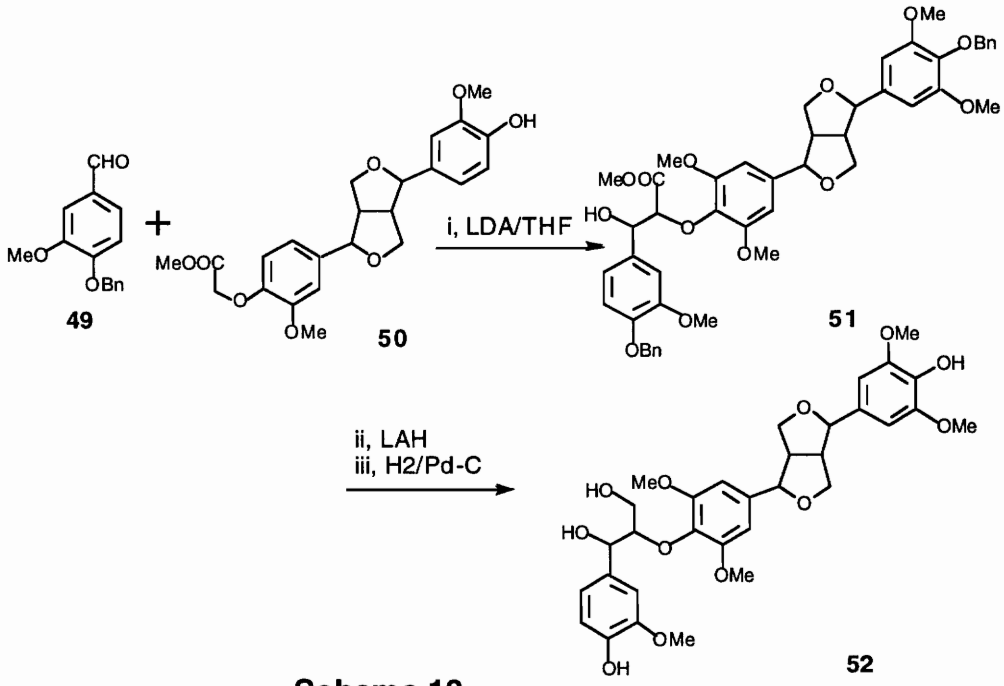


Scheme 11

finally LAH reduction. The synthesis of phenylcoumaran/β-*O*-4 trimer **48** is showed in Scheme 12, with preparation of the β-*O*-4 unit according to the Nakatsubo procedure (Scheme 1). LAH reduction of **47** followed by acidic deprotection of THP and sodium borohydride reduction provided phenylcoumaran/β-*O*-4 trimer **48** in 63% overall yield based on **47**.



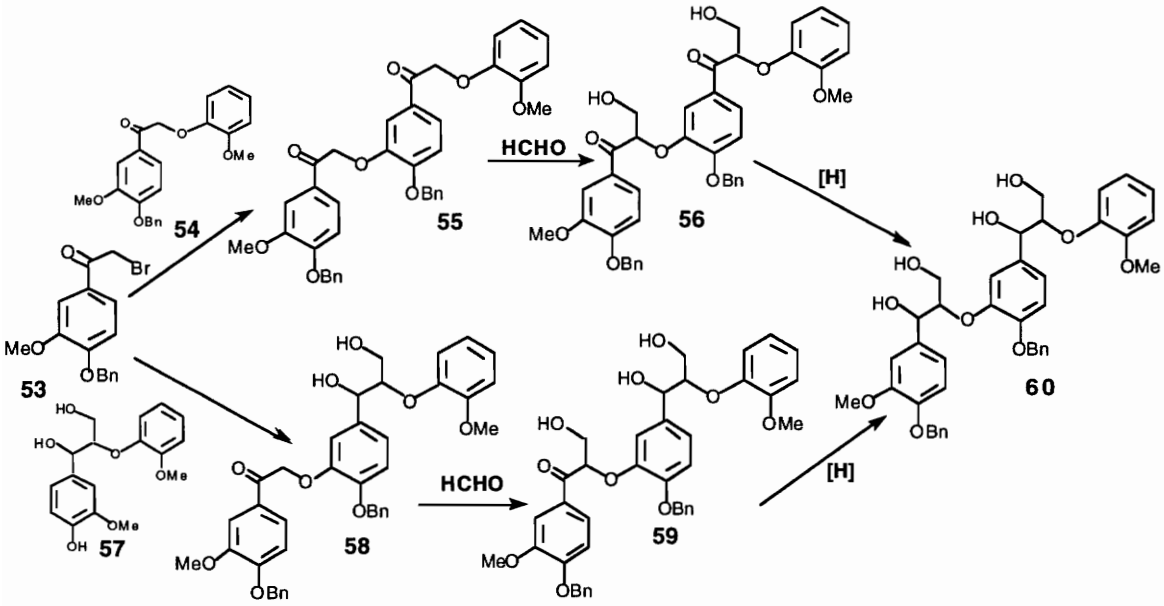
Scheme 12



Scheme 13

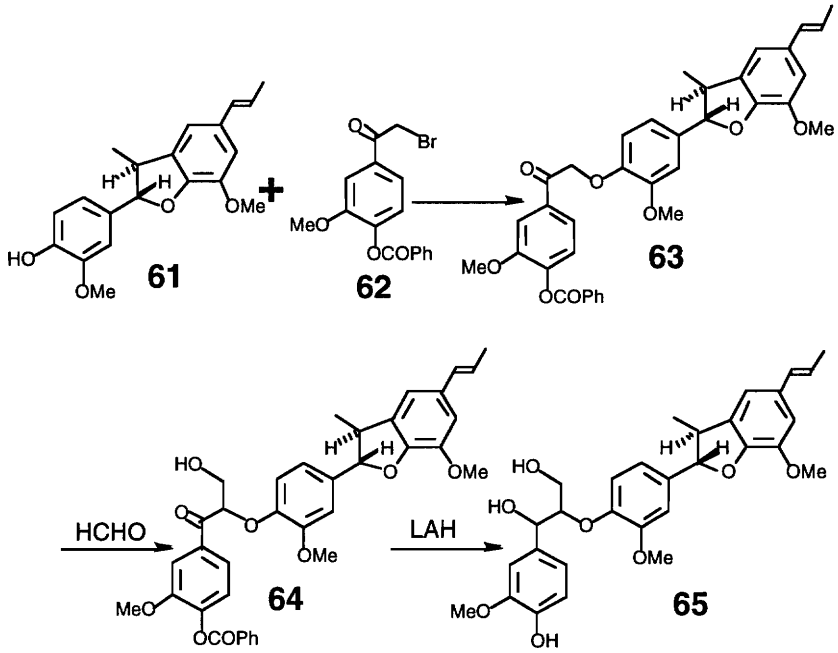
The synthesis of β-*O*-4/syringaresinol trimer **52** is showed in Scheme 13 (Kamaya *et al.*, 1980). Syringaresinol derivative **50** is prepared following protocols outlined in Scheme 5. The aldol type condensation of **49** and **50** provided **51** in about 72% yield. LAH reduction and debenzylation of **51** provided β-*O*-4/syringaresinol trimer **52** in 76% yield.

The β-*O*-4/β-*O*-4 and phenylcoumaran/β-*O*-4 trimers have also been synthesized (Ralph *et al.*, 1986). The synthetic routes are showed in Schemes 14 and 15, respectively. The preparation of β-*O*-4 structure is basically by the Adler procedure (Adler *et al.*, 1955). The bromide **53** can be substituted by different phenols in almost quantitative yields; this easy substitution provides a simple route into a diverse class of trimers having a β-*O*-4 unit linked to another lignin interunit linkage. It was possible to control stereochemistry by means of adding a diastereomerically pure phenol such as the *threo* isomers of **57**, following by stereoselective reduction of α-ketones such as **59**. It is noteworthy that yields in the formaldehyde addition was usually low, for example, 39% from **55** to **56**, and 50-86% from **58** to **59** depending on *threo* or *erythro* isomers of **57** used.



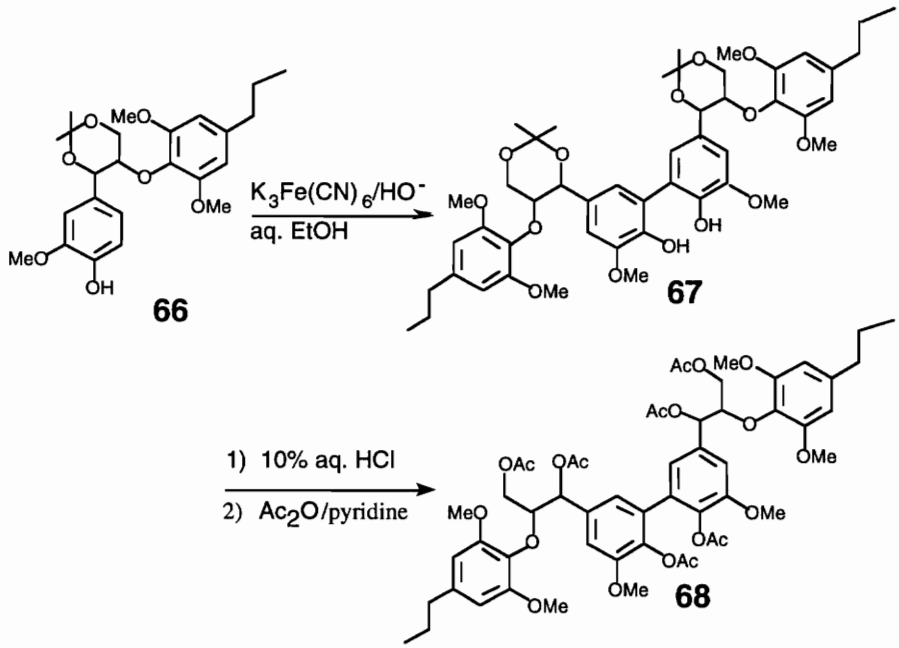
Scheme 14

The synthesis of phenylcoumaran/β-*O*-4 trimer **65** is showed in Scheme 15. Phenylcoumaran **61** was prepared by oxidative coupling of isoeugenol by ferric chloride in 30% yield (Leopold, 1950). Phenol substitution of bromide **62** and subsequent formaldehyde addition provided **64**. LAH reduction of **64** provided the trimer **65**.



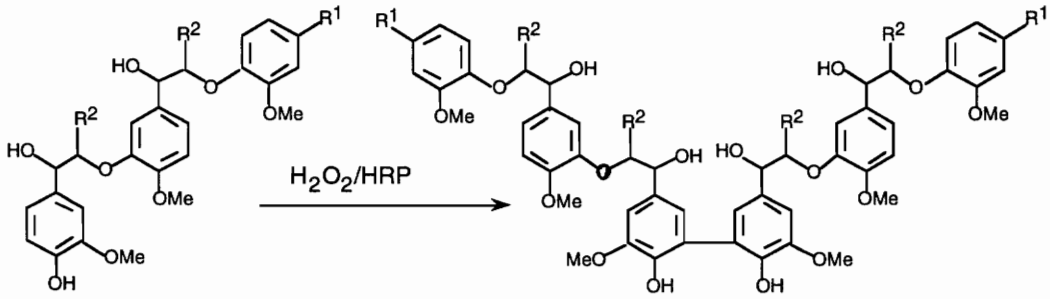
Scheme 15

A β-*O*-4 tetramer peracetate has been synthesized by iterative use of Nakatsubo procedure (Hyatt, 1987). Hyatt (1987) also synthesized a β-*O*-4/5-5' tetramer by a phenolic coupling reaction of an appropriately protected β-*O*-4 dimer (scheme 16). The reaction of phenol **66** with $K_3Fe(CN)_6$ in aqueous NaOH and ethanol provided biphenyl tetramer **67** in 47% yield. Subsequent acid hydrolysis and acetylation provided the peracetylated tetramer **68**. Three different hexameric lignin model compounds have been synthesized (Kilpeläinen *et al.*, 1994a). These hexamers are the highest synthetic oligomers reported so far. The hexamers were prepared by enzymatic oxidation of β-*O*-4 trimers that were prepared according to Nakatsubo procedure (Scheme 17). The oxidation of trimers **69**, **70** and **71**



Scheme 16

with horseradish peroxidase, hydrogen peroxide in aqueous dioxane provide hexamers **72**, **73** and **74**, respectively, in 30-38% yield. The enzymatic oxidative dimerization of trimers is a rapid and convenient way of obtaining a significant amounts of hexamers. These hexamers represent the important β-*O*-4 and 5-5' interunits in lignin. More importantly, the molecular weight of these hexamers are close to those of low molecular



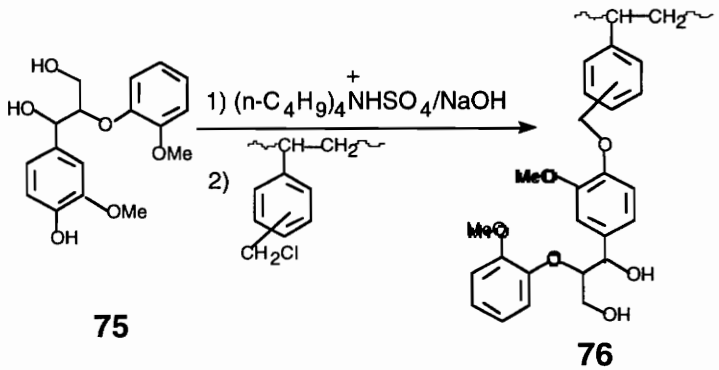
69 $R^1=R^2=H$
70 $R^1=H, R^2=CH_2OH$
71 $R^1=R^2=CH_2OH$

72 $R^1=R^2=H$
73 $R^1=H, R^2=CH_2OH$
74 $R^1=R^2=CH_2OH$

Scheme 17

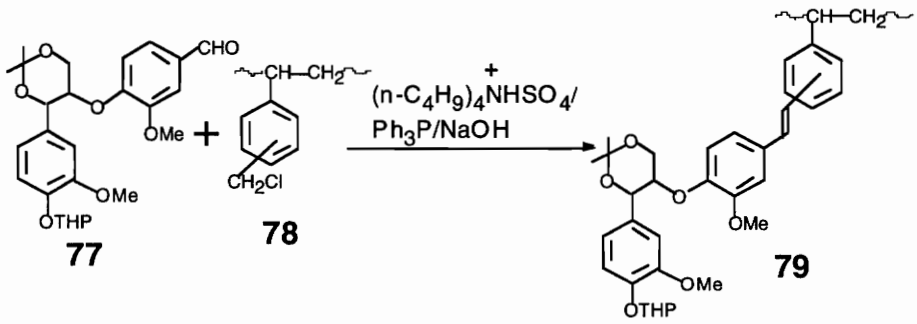
fractions of milled wood lignin and dehydrogenation polymers. Significant amounts of structurally well-defined hexamers are very useful for the study of chemical and biological degradation of lignin.

In order to increase size of model compounds and mimic the polymeric characteristics of lignin, small lignin model compounds have been attached to polystyrene resin. Polystyrene resins are usually chosen because it, like lignin, is a crosslinked aliphatic-aromatic polymer which is stable under pulping conditions. In order to investigate the reactivity of the attached lignin model compounds in polystyrene, stability of the linkage between lignin model compounds and polystyrene in various pulping conditions is very crucial. Connors *et al.* (1977) attached lignin model compounds to polystyrene via esters. This is a good attempt, but ester linkages are not stable under pulping conditions. Apfeld *et al.* (1988) attached the primary hydroxyl in lignin model compounds to polystyrene by a trityl ether linkage. This trityl ether linkage was also found to be unstable under pulping conditions (aqueous alkali at 150 °C). In an effort to prepare the more stable linkages between lignin model compounds and polystyrene resin, lignin model compounds have been attached to polystyrene by benzyl ether linkages. Barkhau *et al.*(1990) attached the lignin-like monomer, guaiacylpropanol to polystyrene through their primaryl hydroxyls. However, these polymer supported lignin model compounds are too simple, not very useful for the reactivity studies. Kilpeläinen *et al.*(1994b) attached a β-*O*-4 dimer to polystyrene through its phenol hydroxyl group (Scheme 18). The nucleophilic reaction was successfully accomplished under phase transfer catalysis.



Scheme 18

Attachment of a β-*O*-4 lignin dimer to polystyrene via a C=C is shown in Scheme 19 (Kilpeläinen and Brunow, 1994). A Wittig reaction was used to attach lignin model compound **77** to chloromethylated polystyrene **78**.



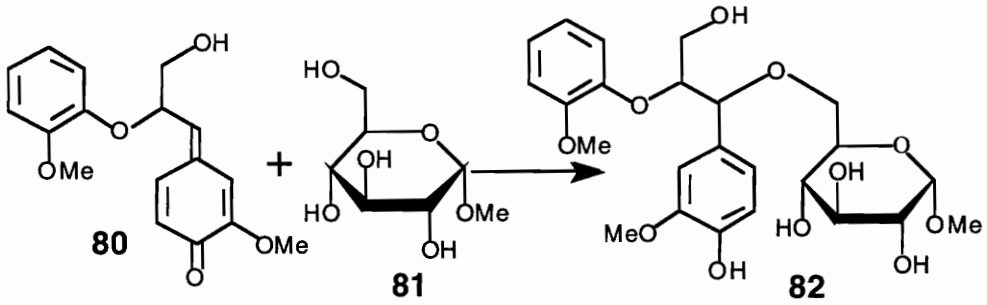
Scheme 19

3.2 Synthesis of lignin-carbohydrate model compounds

The lignin-carbohydrate linkages have been regarded as bridges between hydrophobic lignin and hydrophilic polysaccharides within cell wall. The intimate association between lignin and carbohydrates maybe partly responsible for the conditions required for pulping and bleaching, the reduced digestibility of polysaccharides by ruminants and the relative inefficiency of wood biodegradation. However, the nature of covalent linkages between lignin and carbohydrates such as their types, frequency and functions in cell wall is still poorly understood. The understanding of types, frequency and biosynthetic pathway of LC linkages in cell wall is the prerequisite for the genetic manipulation of plants and postharvest biochemical treatment. The knowledge of the reactivities of LC linkages in chemical and biological conditions would improve wood utilization. But it is difficult to directly gather information about the nature and reactivity of LC linkages from native wood or its isolates because of the complexity of the cell wall and the low frequency of LC linkages. It is very important and indispensable to establish the methods of isolating native LCC without degradation of the linkages and to develop protocols for the selective

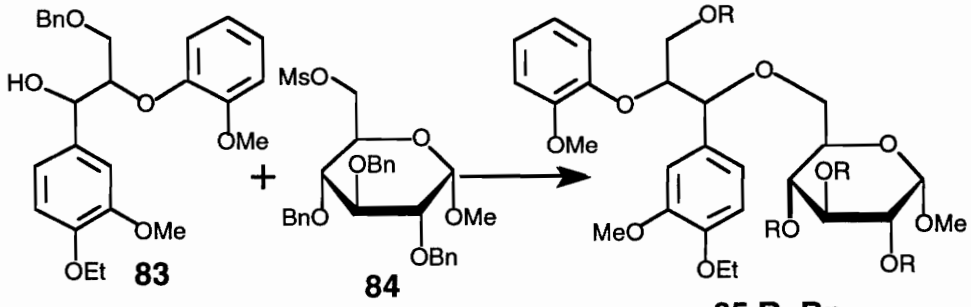
cleavage of specific LC bonds. Lignin-carbohydrate model compounds are therefore very important in the investigation of the nature and reactivity of LC linkages in wood.

According to the mechanism of forming LC linkages, many bonds such as ether, ester and glycoside may exist between lignin and carbohydrates in wood. Freudenberg *et al.* (1959, 1968) first demonstrated that sucrose was incorporated into dehydrogenative polymerization of coniferyl alcohol and proposed that the incorporation was via addition of carbohydrates to quinone methide. Several LC model compounds with ether and ester linkages have been synthesized by this quinone methide addition with carbohydrates. Taneda *et al.* (1987) demonstrated that the reaction of quinone methide **80** with methyl α-D-glucopyranoside **81** provided LC model compound **82** (Scheme 20).



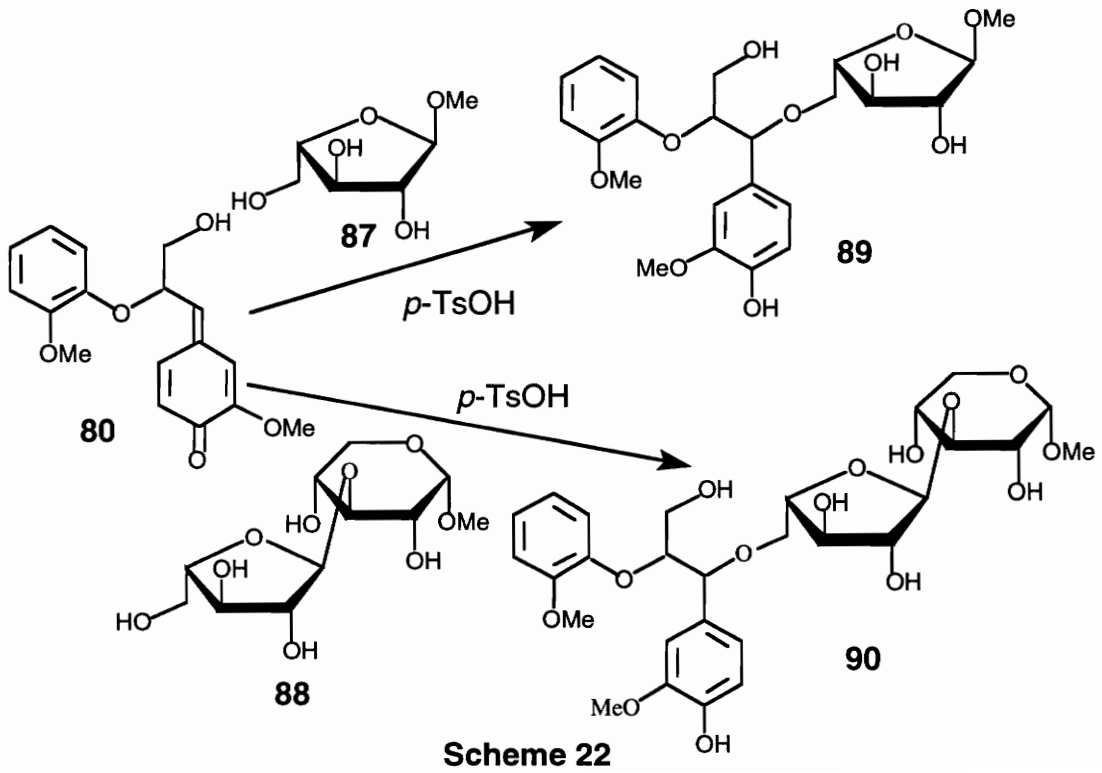
Scheme 20

The primary hydroxyl group is more reactive than secondary hydroxyl groups in methyl α-D-glucopyranoside. Sipilä and Brunow (1991a) later found that the best results could be



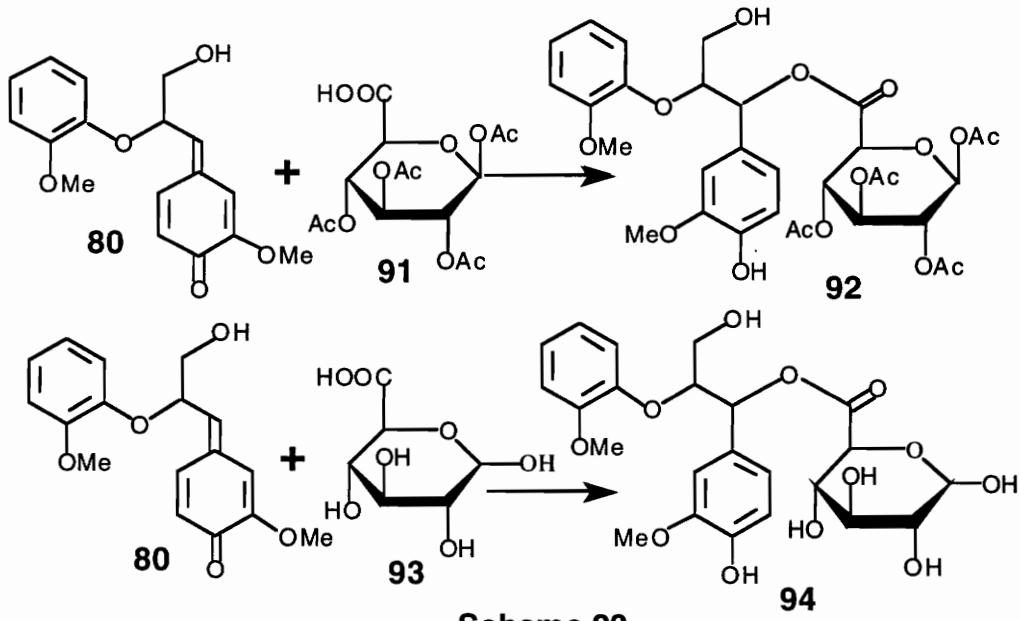
Scheme 21

obtained when the reaction was catalyzed with *p*-toluenesulfonic acid in DMF (about 30% yield). Because of racemic nature of phenylpropane side chain, compound **82** contains four diastereomers which can be detected by HPLC and NMR spectra of peracetylated **82** (Taneda *et al.*, 1987; Sipilä and Brunow, 1991a). The synthesis of the four diastereomers of **82** has recently been reported (Scheme 21) (Tokimatsu *et al.*, 1996). A pair of diastereomers of **85** were synthesized by the reaction of pure *threo* or *erythro* isomers of **83** with **84** in the presence of sodium hydride and trioctylmethylammonium chloride in N,N-dimethylformamide (DMF). The resulting two diastereomers were separated by silica-gel TLC. Hydrogenation of **85** provided **86**. Toikka *et al.* (1994, 1995) synthesized ether-linked LC model compounds **89** and **90** (Scheme 22). It has been found that the reaction of quinone methide **80** with **87** and **88** forms bonds mainly through the primary hydroxyl group.



Scheme 22

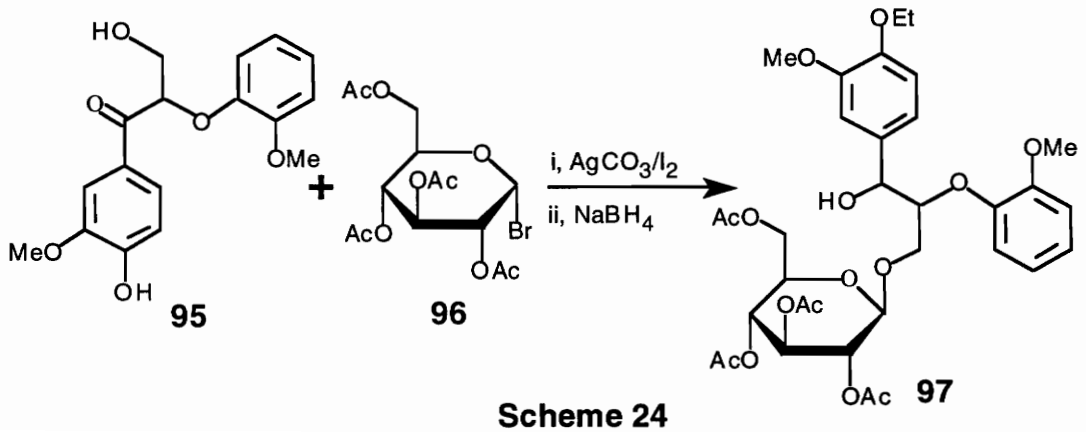
The reactivity of quinone methide depends on the acidity of nucleophiles (Freudenberg, 1968). This implies that the addition of carboxylic acids to quinone methide may also be an important reaction during the biosynthesis of lignin, particularly in the formation of LCCs. The recent discovery that *p*-coumaric acid is attached exclusively at γ-position of lignin sidechain cannot be well explained by the quinone methide mechanism (Ralph *et al.*, 1995). In an effort to better understand the mechanism of forming ester-linked lignin-carbohydrate bonds, several ester-linked LC model compounds have been synthesized. Tanaka *et al.* (1976) demonstrated that the reaction of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosiduronic acid **91** with quinone methide **80** in chloroform provided **92** (scheme 23). It was found that the sole product isolated from the reaction of quinone methide **80** and D-glucuronic acid **93** was compound **94**. The reaction of D-glucuronic acid **93** with quinone methide **80** was also investigated in different reaction conditions such as in the presence of phenols, alcohols and water (Sipilä and Brunow, 1991b).



Scheme 23

The major uronic acid in hardwood hemicelluloses is 4-*O*-methyl-glucuronic acid (Timell, 1967). The ester-linked LC model compounds prepared from 4-*O*-methyl glucuronic acid would more appropriately represent LC ester linkages in hardwood than those prepared from D-glucuronic acid. We have prepared *threo* and *erythro* ester-linked LC model compounds having methyl 4-*O*-methyl-α-D-glucopyranosiduronsyl group attached at α- and γ-positions of phenylpropane side chain (cf. Chapter 6, Li and Helm, 1995).

Glycosides linked to phenol hydroxyl, and the primary hydroxyl at the γ-positon of propane sidechain have been found in wood extractives such as phenylpropane derivatives and lignans. Such glycosidic linkages are very likely present in lignin-carbohydrate complex. It has been demonstrated that glycosidic linkages between the monosaccharides and polyphenol were formed in the *in vitro* synthesis of a DHP-sugar from coniferyl alcohol and a crude enzyme extract from *Populus tremula* in the presence of a large excess of arabinose and glucose (Joseleau and Kesraoui, 1986). Enoki *et al.* (1983) have synthesized several LC model compounds with a glucopyranosidic linkage at γ-position of propane sidechain (Scheme 24). An improved procedure to synthesize γ-glucopyranosides and xylopyranosides has been developed (Helm *et al.*, 1996).

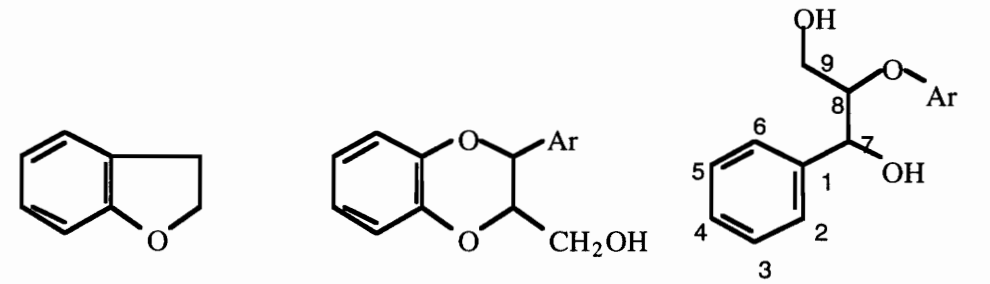


Scheme 24

3.3 Synthesis of Neolignans

A series of reviews dealing with lignans and neolignans have been published (Ward 1995, 1993; Whiting 1990, 1985). The distribution of lignans and neolignans in six families of coniferae has also been reviewed from 1967 to 1994 (Castro *et al.* 1995). While a number of lignans have been synthesized; publications concerning the synthesis of neolignans are relatively few. Published works on neolignans are dominated by the isolation and characterization of new compounds. The synthesis of neolignans are reviewed below.

According to Haworth and Gottlieb's definition (Haworth, 1942; Gottlieb, 1972), lignans imply structures composed of two C_6C_3 units linked by β-β' (8-8'). Neolignans are compounds containing two C_6C_3 units that are linked other than by a β-β' bond. Neolignans are a group of natural products having a very broad structural variation. Arylbenzofurans, benzodioxanes and 8-phenoxypropane (8-*O*-4 neolignans) are three major types of neolignans (Figure 1). Redefinition of lignans and neolignans has been attempted (Gottlieb, 1978). The products of oxidative coupling of allyl or propenyl phenyl



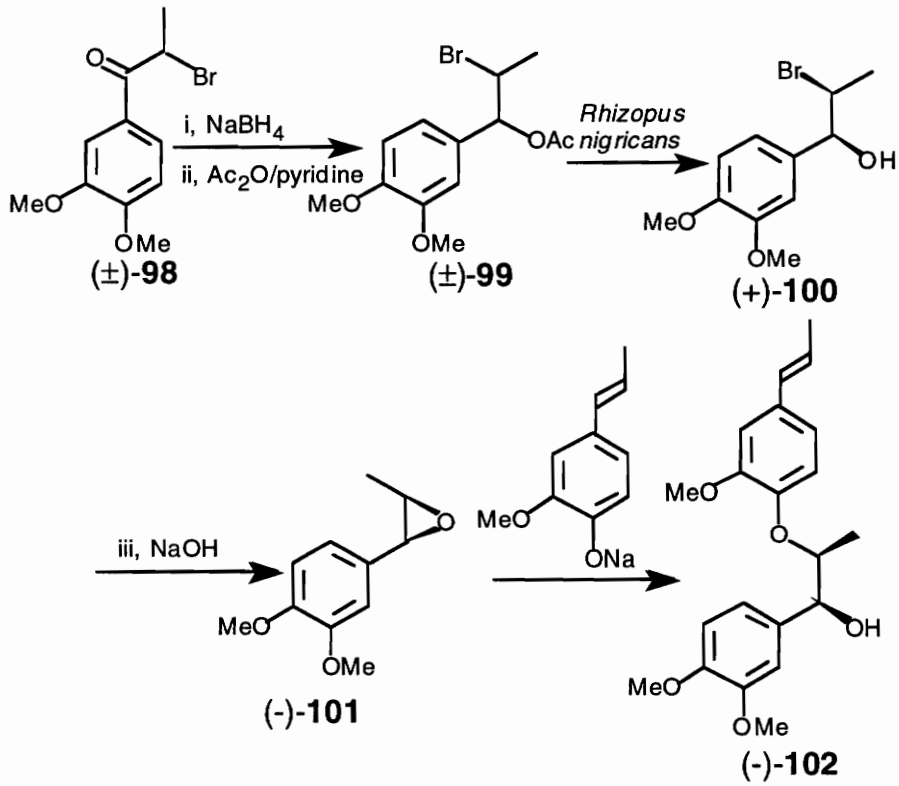
A arylbenzofurans **B** benzodioxanes **C** 8-phenoxypropane

Figure 1

alcohols are regarded as neolignans, while lignans are the coupling products of cinnamyl alcohols. The later definition actually was based on biosynthetic origins of lignans and

neolignans, which are still poorly understood. Haworth and Gottlieb's orginal definition is still widely accepted.

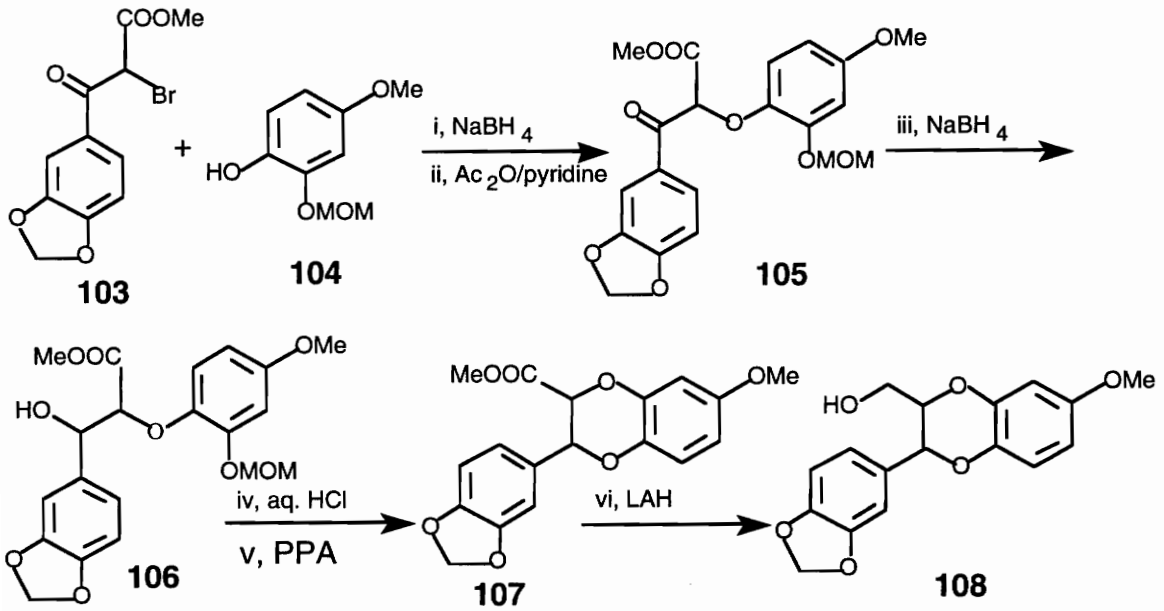
Synthesis of neolignans have been attempted by using biomimetic methods. This is an effort to gain insight into how the various neolignans arise. However, no optically active neolignans have yet been prepared by these methods. Oxidation of isoeugenol, methyl ferulate, and coniferyl alcohol using horseradish peroxidase and hydrogen peroxide or a manganese (III) porphyrin complex affords mainly the corresponding racemic dihydrobenzofurans (Chioccara *et al.* 1993). Quideau and Ralph (1994) demonstrated that the oxidative coupling of (*E*)-coniferyl alcohol with silver (I) oxide provided an arylbenzofuran neolignan in 50% yield.



Scheme 25

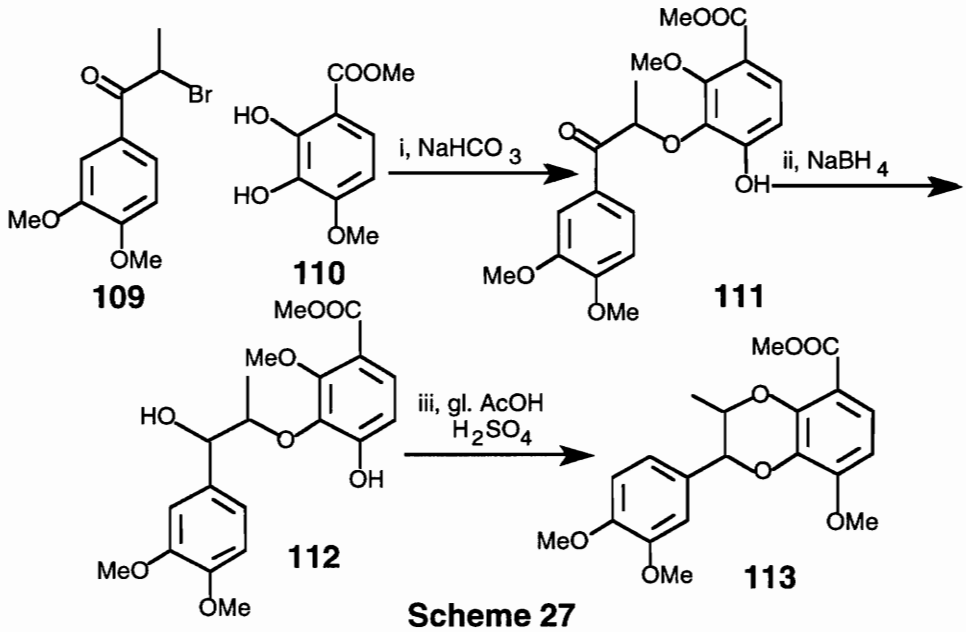
Zanarotti (1983) demonstrated that 8-*O*-4 neolignans (a *threo* and *erythro* mixture) can be prepared by oxidation of 4-(prop-1-enyl)phenols with silver (I) oxide. Zacchino and Badano (1991) prepared *erythro*- 2-(4-allyl-2,5-dimethoxyphenoloxy)-1-hydroxyl-1-(3,4-methylenedioxyphenyl)propane (an 8-*O*-4 neolignan) by using an asymmetric reduction. Zacchino (1994) has also developed an enantioselective route to synthesize (-)-virolin, (-)-**102** (a *threo* 8-*O*-4 type neolignan) (scheme 25). The racemic acetates **99** were selectively hydrolyzed with *Rhizopus nigricans* to provide (+)-**100**. The regiospecific opening of the chiral epoxide (-)-**101** with sodium phenolate to afford (-)-virolin (-)-**102**. We have found that the ring opening of an epoxide analogous to **101** with sodium phenolate provided a mixture without regiospecificy (cf. Chapter 7).

The synthesis of several racemic 1,4-benzodioxane compounds have been reported (Ishibashi and Taniguchi, 1989; Goyal *et al.*, 1993; Arnoldi *et al.*, 1986), but the preparations of optically active 1,4-benzodioxane compounds have not yet been reported.



Scheme 26

Ishibashi and Taniguchi (1989) synthesized the benzodioxane portion **108** of haedoxans (Scheme 26). Goyal *et al.* (1993) prepared a benzodioxane **113** (Scheme 27).



Scheme 27

Arnoldi *et al.*(1986) synthesized several 2-phenylbenzodioxanes as sweeteners. We have developed synthetic methods to prepare chiral 8-*O*-4 neolignans and a chiral benzodioxane (cf. Chapter 8).

3.4 References

Adler, E., *Wood Sci. Technol.*, 11 (**1977**) 169.

Adler, E.; Lindgren, B. O. and Saedén, U., *Svensk Papperstidn.*, 55 (**1952**) 245.

Adler, E. and Eriksoo, E., *Acta. Chem. Scand.*, B 9 (**1955**) 341.

Ahvonen, T.; Brunow, G.; Kristersson P. and Lundquist, K., *Acta. Chem. Scand.*, B 37 (**1983**) 845.

Apfeld, P. B.; Bovee, L. F.; Barkhau, R. A. and Dimmel, D. R., *J. Wood Chem.*

Technol., 8 (**1988**) 483.

Arnoldi, A.; Camarda, L. and Merlini, L., *J. Agric. Food Chem.* 34 (**1986**) 339.

Barkhau, R. A.; Malcom, E. W. and Dimmel, D. R., J. Wood Chem. Technol., 10 (**1990**) 233.

Berndtsson, I.; Khanna, B. L. and Lundquist, K., *Acta. Chem. Scand.*, B 34 (**1980**) 453.

Brezny, R. and Pufflerova, A., *Coll. Czech. Chem. Commun.*, 43 (**1978**) 3263.

Brunow, G.; Sipilä, J.; Lundquist, K. and von Unge, S., *Cellulose Chem. Technol.,* 22 (**1988**) 191.

Brunow, G. and Lundquist, K., *Acta Chem. Scand.* B 38 (**1984a**) 323.

Brunow, G. and Lundquist, K., *Acta Chem. Scand.* B 38 (**1984b**) 335.

Chioccara, F.; Poli, S.; Rindone, B.; Pilati, T.; Brunow, G.; Pietikäinen, P. and Setälä, H., *Acta. Chem. Scand. B*, 47 (**1993**) 610.

Connors, W. J.; Brunow, G. and Kirk, T. K., *TAPPI Conference Papers, Forest Biology Wood Chemistry Conference*, June **1977**, Madison, WI., pp 163.

Enoki, A.; Yaku, F. and Koshijima, T., *Holzforschung*, 37 (**1983**) 135.

Freudenberg, K. In *Constitution and Biosynthesis of Lignin*; Freudenberg, K., Neish, A. C., Eds.; Spring-Verlag, Berlin, **1968**, pp 47-116.

Freudenberg, K. and Grion, G., *Chem. Ber.*, 92 (**1959**) 1355.

Gottlieb, O. R., *Phytochemistry*, 11 (**1972**) 1537.

Gottlieb, O. R., *Fortschr. Chem. Org. Naturst.*, 35 (**1978**) 1.

Goyal, S.; Narula, P. K.; Sharma, P. and Parthasarathy, M. R., *Indian J. Chem., Section B.*, 32 (**1993**) 435.

Haworth, R. D., *J. Chem. Soc.*, (**1942**) 448.

Helm, R. F and Ralph, J., *J. Wood Chem. Technol.,* 13(4) (**1993**) 593.

Helm, R. F. and Li, K., *Holzforschung*, 49 (**1995**) 533.

Helm, R. F.; Toikka, M.; Li, K. and Brunow, G., *J. Chem. Soc. Perkin Trans. 1*, (**1996**) in press.

House, H. O. and Ryerson, G. D., *J. Am. Chem. Soc.*, 83 (**1961**) 979.

Hyatt, J. A., *Holzforschung*, 41 (**1987**) 363.

Ibrahim, W. and Lundquist, K., *Acta Chem. Scand.* B 48 (**1994**) 149.

Ichihara, A.; Numata, T.; Kanai, S. and Sakamura, S. *Agric. Biol. Chem.*, 41 (**1977**) 1813.

Ichihara, A.; Oda, K.; Numata, Y. and Sakamura, S., *Tetrahedron Lett.*, 19 (**1978**) 3035.

Ichihara, A.; Kanai, S.; Nakamura, Y. and Sakamura, S., *Tetrahedron Lett.*, 17 (**1976**) 3961.

Ishibashi, F. and Taniguchi, E., *Agric. Biol. Chem.*, 53 (**1989**) 1557.

Joseleau, J-P. and Kesraoui, R., *Holzforshung*, 40 (**1986**) 163.

Kamaya, Y.; Nakatsubo, F. and Higuchi, T. *Mokuzai Gakkaishi*, 26 (**1980**) 471.

Karhunen, P.; Rummakko, P.; Sipilä, J.; Brunow, G. and Kilpeläinen, I., *Tetrahedron Lett.*, 36 (**1995a**) 4501.

Karhunen, P.; Rummakko, P.; Sipilä, J.; Brunow, G. and Kilpeläinen, I., *Tetrahedron Lett.*, 36 (**1995b**) 169.

Katayama, Y. and Fukuzumi, T., *Mokuzai Gakkaishi*, 24 (**1978**) 664.

Kilpeläinen, I.; Tervilä-Wilo, A.; Peräkylä, H.; Matikainen, J. and Brunow, G., *Holzforschung*, 48 (**1994a**) 381.

Kilpeläinen, I.; Tervilä-Wilo, A. and Brunow, G., *Holzforschung*, 48 (**1994b**) suppl. 59.

Kilpeläinen, I. and Brunow, G., *Holzforschung*, 48 (**1994**) 222.

Kristersson, P. and Lundquist, K., *Acta Chem. Scand.* B 34 (**1980**) 213.

Landucci, L. L., *J. Wood Chem. Technol.*, 15 (**1995**) 349.

Leopold, B., *Acta Chem. Scand.* 4 (**1950**) 1523.

Li, K. and Helm, R. F., *J. Agric. Food Chem.*, 43 (**1995**) 2098.

Lundquist, K. and Miksche, G. E., *Tetrahedron Lett.*, (**1965**) 2131.

Lundquist, K. and Remmerth, S., *Acta Chem. Scand.,* B 29 (**1975**) 276.

Miki, K.; Sasaya, N. and Sakakibara, A., *Abstract of 29th Annual Meeting of Japan Wood Research Soc.*, (**1979**) 333.

Nakatsubo, F. and Higuchi, T., *Mokuzai Gakkaishi* , 25 (**1979**) 735.

Nakatsubo, F. and Higuchi, T., *Mokuzai Gakkaishi* , 26 (**1980a**) 107.

Nakatsubo, F. and Higuchi, T., *Mokuzai Gakkaishi*, 26 (**1980b**) 31.

Nakatsubo, F.; Sato, K. and Higuchi, T., *Holzforschung*, 29 (**1975**) 165.

Nakatsubo, F. and Higuchi, T., *Holzforschung*, 29 (**1975**) 193.

Nonni, A. J, and Dence, C. W., *J. Wood Chem. Technol.* 2 (**1982**) 161.

Pearl, I. A. and Gratzl, J., *J. Org. Chem.,* 27 (**1962**) 2111.

Popoff, T. and Theander, O., *Phytochemistry*, 14 (**1975**) 206.

Popoff, T. and Theander, O., *Acta Chem. Scand.*, B 31 (**1977**) 329.

Quideau, S. and Ralph, J., *Holzforschung* , 481 (**1994**) 12.

Ralph, J., *Holzforschung*, 42 (**1988**) 273.

Ralph, J.; Hatfield, R. D.; Quideau, S; Helm, R. F.; Grabber, J. H. and Jung, H-J. G., *J. Am. Chem. Soc.,* 116 (**1994**) 9445.

Ralph, J. and Helm. R. F., *J. Agric. Food Chem.*, 39 (**1991**) 705.

Ralph, J. and Young. R.A., *Holzforschung*, 35(1) (**1981**) 39.

Ralph, J.; Ede, R. M. and Wilkins, A. L., *Holzforschung*, 40 (**1986**) 23.

Sarkanen, K. V. and Wallis, A. F. A., *J. Chem. Soc., Perkin Trans. 1*, (**1973**) 1869.

Sipilä, J. and Syrjänen, K., *Holzforschung*, 49 (**1995**) 325.

Sipilä, J. and Brunow, G., *Holzforshung*, 45 (**1991a**) suppl. 3.

Sipilä, J. and Brunow, G., *Holzforshung*, 45 (**1991b**) suppl. 9.

Takemoto, T.; Miyase, T. and Kusano, G., *ibid.*, 14 (**1975**) 1890.

Tanaka, K., Nakatsubo, F. and Higuchi, T., *Mokuzai Gakkaishi* , 22 (**1976**) 589.

Taneda, H.; Nakano, J.; Hosoya, S. and Chang, H-M., *J. Wood Chem. Technol.*, 7 (**1987**) 485.

Timmel, T. E., *Wood Sci. Technol.*, 1 (**1967**) 45.

Toikka, M.; Brunow, G.; Sipilä, J.; Teleman, A. and Helm, R. F., *Third European Workshop on Lignocellulosics and pulp.* Stockholm, August 28-31, **1994**, 183.

Toikka, M.; Brunow, G. and Teleman, A., *The 8th International Symposium on Wood and pulping Chemistry.* Helsinki, Finland, June **1995**, vol.1, 147.

Tokimatsu, T.; Umezawa, T. and Shimada, M., *Holzforshung*, 50 (**1996**) 156.

Wallis, A. F. A., *Aust. J. Chem.*, 26 (**1973**) 585.

Ward, R. S., *Nat. Prod. Rep.*, 12 (**1995**) 183.

Ward, R. S., *Nat. Prod. Rep.*, 10 (**1993**) 1.

Whiting, D. A., *Nat. Prod. Rep.*, 7 (**1990**) 349.

Whiting, D. A., *Nat. Prod. Rep.*, 4 (**1987**) 499.

Zacchino, S. A. and Badano, H., *J. Nat. Prod.*, 54 (**1991**) 155.

Zacchino, S. A., *J. Nat. Prod.*, 57 (**1994**) 446.

Zanarotti, A., *J. Chem. Res. (M)*, (**1983**) 2625.

Chapter 4

Complete *Threo*-Stereoselectivity for the Preparation of β-*O*-4 Lignin Model Dimers

4.1 Abstract

Reduction of the α-ketone of several β-*O*-4 lignin model precursors with the asymmetric reductant diisopinocampheylchloroborane (DIP-chloride™) provides the *threo* isomers in 80% yield and greater than 98% purity. Although this reductant is available in two chiral forms, no enantioselectivity was observed.

4.2 Introduction

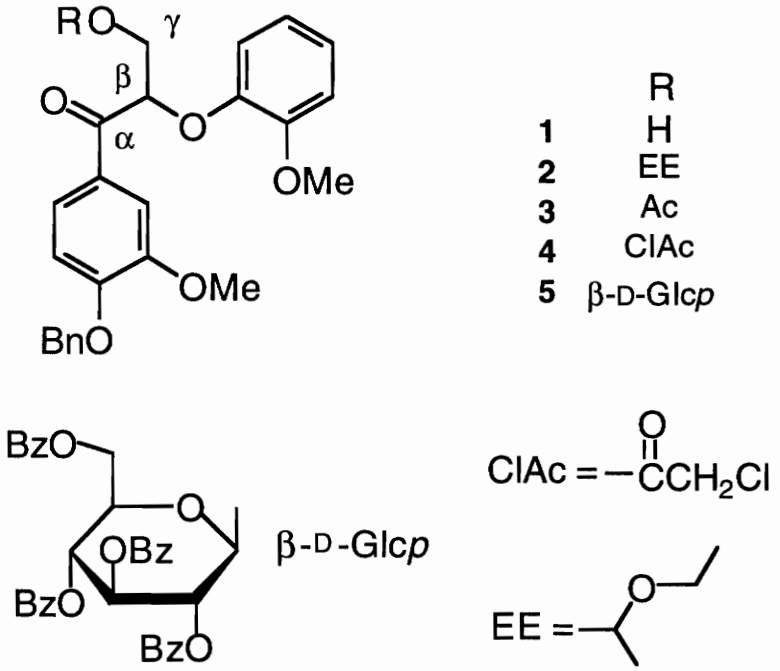
It is well established that the most predominant interunit linkage present in lignin is the so-called β-*O*-4 linkage (Adler 1977; Fengel and Wegener 1989; Eberhardt *et al.* 1993). The stereochemistry about the side chain of β-*O*-4 units has been the subject of numerous investigations (for example, see: Lundquist 1980; Hauteville *et al.* 1986; Brunow *et al.* 1993), and it appears at present that if nucleophilic attack of the intermediate quinone methide by water is a random (non-enzymatic) reaction, the *threo:erythro* ratio is dependent mostly on sterics and the pH of the reaction medium (Brunow *et al.* 1993). This then affords a mixture of racemic *threo* and *erythro* isomers which adds a significant degree of complexity to the NMR chemical shift range of β-*O*-4 ethers (Ralph 1993), oftentimes making assignments of native tissue spectra possible only through the use of 2D-NMR techniques. The matter is further complicated by considering the attachment of chiral hemicellulose to lignin, which makes the NMR spectra of native lignin-carbohydrate complexes even more complicated. If one wishes to ascertain the regiochemistry of lignin-

carbohydrate complexes in wood through the use of NMR spectroscopy, techniques for the preparation and characterization of optically active lignin and lignin-carbohydrate (LC) complexes becomes imperative.

Our laboratory is currently interested in determining the regiochemical aspects of lignin-carbohydrate (LC) covalent interactions in plant cell walls. As an NMR database of suspected LC models is needed, we have been investigating the use of chiral reductants for the preparation of optically active synthetic LC models. We have chosen the β-*O*-4 model as the most likely site of carbohydrate attachment. Carbohydrates can theoretically be attached to lignin β-*O*-4 units at the α-position, via direct attack of the intermediate quinone methide, or to the free phenolic and/or γ-positions, by enzyme-mediated reactions (for an example of an enzyme mediated lignin modification see Ralph *et al.* 1994). Utilizing the synthetic methodology originally described by Adler (Adler and Eriksoo 1955), it has been shown that high *erythro*-selectivity (>95%) can be obtained by reduction of β-*O*-4 lignin model α-ketones with zinc borohydride (Helm and Ralph 1993). *Threo*-selectivity for reduction of the ketone is currently limited to about 80% with the use of water:alcohol solutions of sodium borohydride (Barrelle *et al.* 1989; Ralph and Helm 1991). It should be noted that both *threo* and *erythro* forms are available via an alternative route which utilizies borate complexation for resolution of the isomeric racemates (Ibrahim and Lundquist 1994). We wish to report here that extremely high *threo*-selectivity (>98%) can now be obtained by reducing the α-ketone with the asymmetric reductant diisopinocampheylchloroborane (DIP-chloride™). Although this commercially-available chiral reductant is available in two enantiomeric forms, we have found that reduction of lignin model precursors (racemic at the β-postion) with either form affords both *threo* isomers almost exclusively. Thus the combination of zinc borohydride and DIP-chloride allows entry into pure (>95%) *threo* and *erythro* isomers; allowing us to address the synthetic challenges involved in the preparation of accurate LC models.

4.3 Results and Discussion

Reductions were performed on **1**-**5**. Protection of the γ-hydroxyl with a relatively acid-stable protecting group is required as reductions of **1** and **2** were not successful. Yields for **3**-**5** were in the 80% range with the *erythro* isomer not detectable in the ^{1}H-NMR spectra (Fig. 1). While the acetate was stable towards acid-catalyzed migration to the α-position, the chloroacetate group slowly migrated to the α-position. This migration from a primary to benzylic hydroxyl has been observed



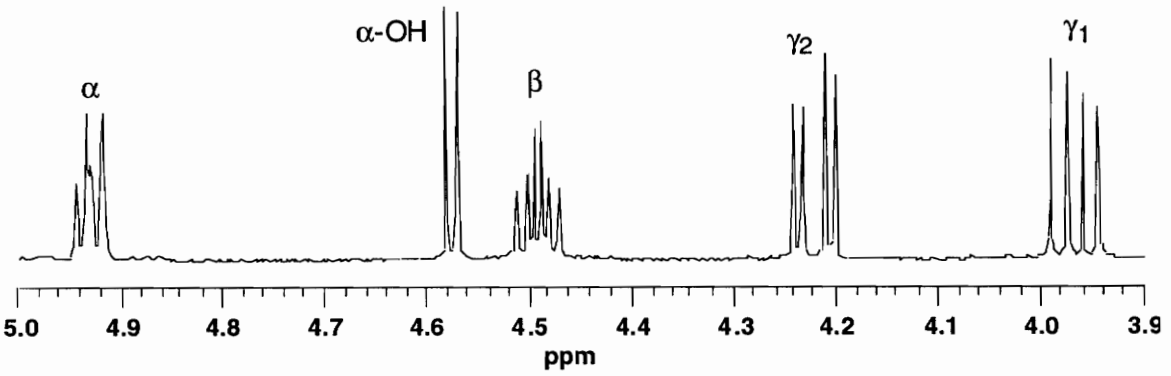


Fig. 1. A portion of the 1H-NMR spectrum of the DIP-Cl reduced **3** in acetone-*d*6 showing the side chain protons. No signals from the two γ-protons of the *erythro* isomers (ca. 4.2—4.4 ppm) were detected.

for acetates with the zinc borohydride reductant (Helm and Ralph, 1993) as well as with γ-uronates (unpublished results).

Glycosylation of a carbohydrate on the γ-position of the lignin model precursors results in a pair of epimers due to the racemic nature of the β-position. Thus reduction of **5** is useful for the direct analysis of the *enantioselective* nature of the reaction as well as for the preparation of synthetic LC models. NMR analysis of the reduction product of this

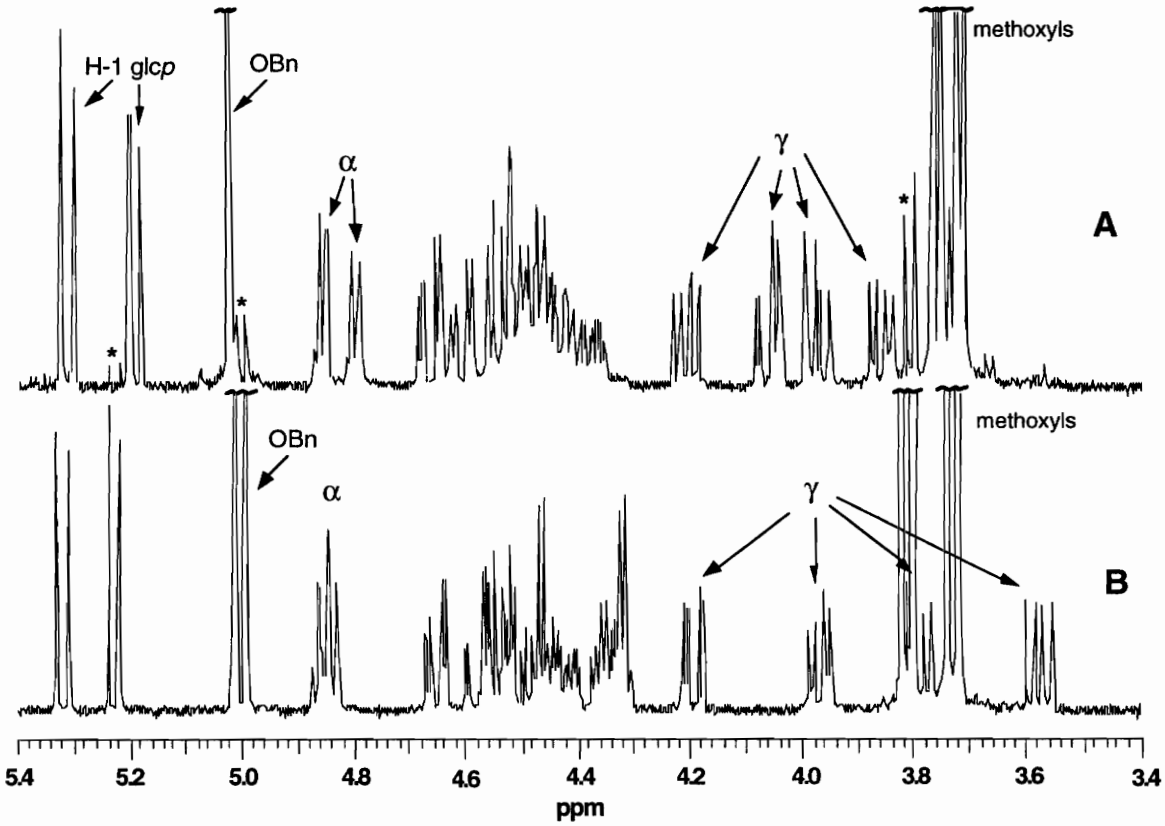


Fig. 2. Portions of the 1H-NMR spectra of zinc borohydride (A) and (+)-DIP-Cl (B) reduced **5** recorded in acetone-*d*6. Asterisks in spectrum A indicate the weak *threo* resonances resulting from incomplete stereoselectivity (*threo:erythro,* 8:92) for the borohydride reduction.

material reveals no enantioselectivity, regardless of which chiral reductant was used as can be seen by the 1:1 mixture of products in Figure 2. This result implies that the reaction mechanism for the reduction is somewhat different than that proposed by Brown and co-workers (Brown *et al.* 1988), and is probably similar to that observed for zinc borohydride (Helm and Ralph 1993), with a reversal of the stereochemical outcome. Additional studies into the mechanism of the reduction, as well as the techniques for the preparation of γ-glycosides will be the subject of future reports.

4.4 Experimental

The general chemical and spectroscopic techniques used in this study have been described (Ralph *et al.* 1992). All NMR experiments were performed with a 400 MHz instrument at room temperature (unless indicated otherwise) with acetone-d_6 as solvent and internal reference (central solvent peak, δ_H, 2.04 ppm; δ_C, 29.8 ppm). Coupling constants are based on a first-order analysis. Compounds **1-3** were prepared by established procedures (Helm and Ralph 1993) and the preparation of **5** will be described elsewhere.

1-(4-benzyloxy-3-methoxyphenyl)-3-chloroacetoxy-2-(2-methoxyphenoxy)propanone (**4**). Crystalline **1** (505.1 mg, 1.24 mmol) was dissolved in CH_2Cl_2 (6 mL) and 4-dimethylaminopyridine (DMAP, 180 mg, 1.48 mmol) and chloroacetyl chloride (110 μL, 1.36 mmol) were added. Thin-layer chromatography (TLC, 19:1 $CHCl_3$:EtOAc) indicated the reaction was complete almost immediately. Methanol was added to quench the reaction and the product was diluted with CH_2Cl_2 in a separatory funnel and washed with cold aq. 3% HCl (4x) followed by aq. NH_4Cl (2x). Standard processing afforded a syrup which was purified by silica gel ($CHCl_3$ then 19:1 $CHCl_3$:EtOAc) to afford **4** as a clear syrup (574.0 mg, 95.6%). ^{1}H NMR δ (ppm): 4.23 (2H, s, $ClCH_2$), 4.61 (1H, dd, J = 6.7

and 11.9 Hz, γ^1), 4.73 (1H, dd, J = 3.8 and 11.9 Hz, γ^2), 5.84 (1H, dd, β); ^{13}C NMR δ (ppm): 41.23 ($ClCH_2$), 66.13 (γ), 79.68 (β) and 193.9 (α).

General procedure for the reduction of α-ketone lignin models. The procedure is essentially that of Brown and co-workers (Brown *et al.* 1988). An oven-dried 10 mL flask was cooled to room temperature under a stream of nitrogen. (+)-DIP-Cl™ or (-)-DIP-Cl™ (available from Aldrich, ca. 2 molar equivalents) was transferred to the flask under nitrogen atmosphere and sealed with a septum, then dissolved in THF (2 mL, distilled from sodium and benzophenone). The solution was cooled to -30 °C with stirring, and a solution of the α-ketone (100 mg) in THF (1 mL) was added. After 30 min, the flask was placed in a freezer (-20 °C), and the reaction was monitored occasionally by TLC ($CHCl_3$-EtOAc, 19:1). When the reaction was almost complete (24 hr), THF was removed under diminished pressure. The resulting syrup was then dried under high vacuum for about two hours. The residue was dissolved in ethyl ether, and diethanolamine (ca. 4 eq) was added. A white gel formed quickly, which then fell out as a solid. After two hours the solid was filtered off and washed with hexane. The combined filtrate was concentrated and further purified via silica gel column chromatography ($CHCl_3$:EtOAc, 19:1, to remove the reduction byproducts, then $CHCl_3$:EtOAc, 1:1, to elute the desired material). Yields ranged from 77-87%. Larger-scale reactions (500 mg of α-ketone) have been performed without difficulty and typically provide higher yields (87%).

Reduction of ketone 3. Standard reduction and workup followed by silica gel chromatography afforded the *threo* isomers of 3-acetoxy-1-(4-benzyloxy-3-methoxyphenyl)-1-hydroxy-2-(2-methoxyphenoxy)propane as a syrup. ^{1}H NMR δ (ppm): 3.99 (1H, dd, J = 6.4 and 11.9 Hz, γ^1), 4.24 (1H, dd, J = 3.5 and 11.9 Hz, γ^2), 4.51 (1H, dt, J = 3.5 and 6.2, β), 4.61 (d, J = 4.0 Hz, α-OH), 4.94 (1H, dd, J = 4.1 and

6.0 Hz, α); ^{13}C NMR δ (ppm): 64.08 (γ), 73.56 (α), 84.07 (β).

Reduction of ketone 4. Standard reduction and workup followed by silica gel chromatography afforded the *threo* isomers of 1-(4-benzyloxy-3-methoxyphenyl)-3-chloroacetoxy-1-hydroxy-2-(2-methoxyphenoxy)propane as a syrup. ^{1}H NMR δ (ppm): 4.11 (1H, dd, J = 6.2 and 11.8 Hz, γ^{1}), 4.20 (2H, s, $ClCH_2$) 4.39 (1H, dd, J = 3.4 and 11.8 Hz, γ^{2}), 4.54 (1H, dt, J = 3.4 and 6.1 Hz, β), 4.67 (d, J = 4.0 Hz, α-OH), 4.98 (1H, dd, J = 4.0 and 6.0 Hz, α); ^{13}C NMR δ (ppm): 41.44 ($ClCH_2$), 65.57 (γ), 73.42 (α), 83.97 (β).

Reduction of ketone 5. Standard reduction and workup followed by silica gel chromatography afforded the *threo* diastereomers of 3-(4-benzyloxy-3-methoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)propyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside as a syrup. Complete spectral characterization (by diastereomer) is not possible at this time, but partial data are as follows (the subscripts I and II indicate individual isomers): ^{1}H NMR δ (ppm, 27 °C): 3.59 (1H, dd, J = 6.0 and 11.1, $\gamma^{1,I}$) 3.79 ($\gamma^{1,II}$), 3.98 (1H, dd, J = 4.0 and 11.6 Hz, $\gamma^{2,II}$), 4.20 (1H, dd, J = 3.1 and 11.1 Hz, $\gamma^{2,I}$), 4.84-4.89 (m, α's), 5.23 (1H, d, J = 7.9 Hz, H-1 of β-D-Glc*p*), 5.32 (1H, d, J = 7.9 Hz, H-1 of β-D-Glc*p*); ^{13}C NMR δ (ppm): 68.59 and 69.40 (γ), 73.11 and 73.19 (α), 85.70 and 85.85 (β), 101.68 and 101.83 (C-1).

4.5 References

Adler E. and E. Eriksoo. 1955. Guaiacylglycerol and its β-guaiacyl ether. Acta Chem. Scand. 9: 341-2.

Adler E. 1977. Lignin chemistry—past, present and future. Wood Sci. Technol. 11:

169-218.

Barrelle M., C.B. Béguin and F. Tetaz. 1989. NMR conformational investigation of stereocontrolled reduction of lignin-model compounds. Holzforschung 43: 339-343.

Brown, H. C., J. Chandrasekharan and P.V. Ramachandran. 1988. Chiral synthesis via organoboranes. 14. Selective reductions. 41. Diisopinocampheylchloroborane, an exceptionally efficient chiral reducing agent. J. Am. Chem. Soc. 110: 1539-1546.

Brunow, G., O. Karlsson, K. Lundquist and J. Sipilä. 1993. On the distribution of the diastereomers of the structural elements in lignins: the steric course of reactions mimicking lignin biosynthesis. Wood Sci. Technol. 27: 281-286.

Eberhardt, T. L., M. A. Bernards, L. He, L. B. Davin, J. B. Wooten and N. G. Lewis. 1993. Lignification in cell suspension cultures of *Pinus taeda*. *In situ* characterization of a gymnosperm lignin. J. Biol. Chem. 268: 21088-21096.

Fengel, D. and G. Wegener. 1989. Wood: chemistry, ultrastructure, reactions. Walter de Gruyter and Sons, Berlin. pp. 132-183.

Hautville, M., K. Lundquist and S. von Unge. 1986. NMR studies on lignins. 7. 1H-NMR spectroscopic investigation of the distribution of *erythro* and *threo* forms of β-*O*-4 structures in lignins. Acta Chem. Scand. B40: 31-35.

Helm, R. F. and J. Ralph. 1993. Stereospecificity for the zinc borohydride reduction of α-aryloxy-β-hydroxy ketones. J. Wood Chem. Technol. 13: 593-601.

Ibrahim, W. and K. Lundquist. 1994. Synthesis of *erythro* and *threo* forms of lignin models of the arylglycerol β-glycerol ether type. Acta Chem. Scand. 48: 149-151.

Lundquist, K. 1980. NMR studies of lignins. 4. Investigation of spruce lignin by 1H NMR spectroscopy. Acta Chem. Scand. B34: 21-26.

Ralph, J. 1993. 1H NMR of acetylated β-ether/β-ether lignin model trimers. Magn. Reson. Chem. 31: 357-363.

Ralph, J. and R. F. Helm. 1991. Rapid proton NMR method for determination of *threo:erythro* ratios in lignin model compounds and examination of reduction stereochemistry. J. Agric. Food Chem. 39: 705-709.

Ralph, J., R. F. Helm, S. Quideau and R. D. Hatfield. 1992. Lignin-feruloyl ester cross-links in grasses. Part 1. Incorporation of feruloyl esters into coniferyl alcohol dehydrogenation polymers, J. Chem. Soc., Perkin Trans. 1. 2961-2969.

Ralph, J., R. D. Hatfield, S. Quideau, R. F. Helm, J. Grabber and H.-J. G. Jung. 1994. Pathway of *p*-coumaric acid incorporation into maize lignin as revealed by NMR. J. Am. Chem. Soc. 116: 9448-9456.

Chapter 5

A practical synthesis of methyl 4-*O*-methyl α-D-glucopyranosiduronic acid

5.1 Abstract

Methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid can conveniently be prepared from methyl α-D-glucopyranoside in 4 steps (74% overall yield). The use of benzyl α-D-glucopyranoside allows entry into the 4-*O*-alkyl-D-glucopyranoses and D-glucuronic acid, and the oxidation methodology should also be suitable for the synthesis of uronosyl-containing oligosaccharides.

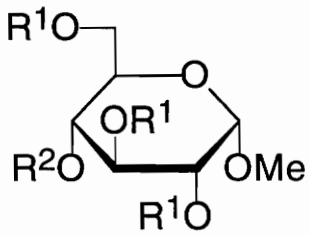
5.2 Introduction

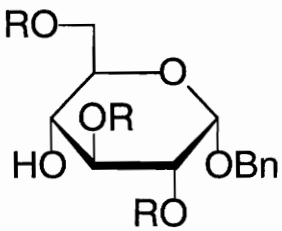
The principal hemicellulose of temperate zone hardwoods is *O*-acetyl-(4-*O*-methylglucurono)xylan, constituting 15-30% of the weight of these woods[1]. The (1→4) β-D-xylopyranosyl backbone carries occasional substitutions at the 2-position by 4-*O*-methyl-α-D-glucopyranosiduronic acid, as well as randomly distributed acetyl groups. The overall structure of xylan in temperate zone hardwoods is quite conserved[2]. This implies that the chemical characteristics of this hemicellulose plays an integral role in hardwood cell wall composite structure. As part of our efforts to more fully understand woody cell wall structure and function, a simple, high yielding route to alkyl 4-*O*-methyl-α-D-glucopyranosiduronic acids from D-glucose was desired.

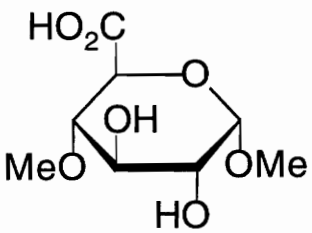
Several synthetic methods have been developed to synthesize methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid and methyl (methyl 4-*O*-methyl-α-D-glucopyranosid)uronate,[3-6] and a chromatographic/chemical method has been used to isolate 4-*O*-methyl-D-glucuronic acid from wood.[7] Since all reported methods involved several steps, the exploration of alternative strategies was deemed worthwhile. We now wish to report a 4-step synthesis of methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid (**7**) from methyl α-D-glucopyranoside (**1**).

5.3 Results and discussion

Methyl α-D-glucopyranoside (**1**) was activated by bis(tributyltin) oxide and subsequently tribenzoylated to give methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside. Slight modifications of the original procedure[8] have improved the yield of **2** from 63 to 90% (see Experimental). Furthermore, benzyl α-D-glucopyranoside (**5**) was also tribenzoylated to afford benzyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**6**) in 83% yield. Compound (**6**) is an excellent precursor for the synthesis of compounds in which the anomeric protecting group needs to be easily removed.







	R^1	R^2
1	H	H
2	Bz	H
3	Bz	Me
4	H	Me

5 R = H
6 R = Bz

7

Compound (**2**) was methylated with methyl triflate in dichloromethane with 2, 6-di-*tert*-butyl-4-methylpyridine[9] to yield methyl 2,3,6-tri-*O*-benzoyl-4-*O*-methyl-α-D-glucopyranoside (**3**) (92% yield). This procedure is only suitable for small scale reactions (for example, ^{13}C-labeled compounds) since it requires a large excess of 2,6-di-*tert*-butyl-4-methylpyridine. Large-scale methylation reactions were performed with iodomethane and silver oxide in DMF, which afford **3** in 80% yield[10,11]. Subsequent debenzoylation of **3** in methanol with catalytic amount of sodium methoxide quantitatively gave **4**.

The selective oxidation of **4** to **7** was achieved after investigation into several oxidation procedures. It has recently been reported that methyl α-D-glucopyranoside can be oxidized with Pt/C, O_2 to give sodium (methyl α-D-glucopyranosid)uronate in very high yield[12]. However, in our hands, the oxidation of **4** with Pt/C, O_2 gave **7** in only about 50% yield. It should be noted that the conversion of **4** to **7** with Pt/C, O_2 has previously been reported to be ineffective[6]. This suggests that the 4-*O*-methyl group changes the reactivity of methyl α–D-glucopyranoside towards oxidation of the primary hydroxyl. The oxidation of **4** with nitrogen dioxide[3] also gave a low yield of **7**.

Our attention was then turned to reports that TEMPO-catalyzed (TEMPO=2,2,6,6-tetramethyl-1-piperidinyloxy) oxidations are very effective for selective oxidation of the primary carbohydrate hydroxyls[13,14]. The reaction of **4** with TEMPO, sodium bromide, and sodium hypochlorite in water at pH=10-11 quickly produced **7** in 90% yield[14].

Thus, in conclusion, methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid can conveniently be prepared from **1** in 4 steps (74% overall yield). The use of benzyl α-D-glucopyranoside (**5**) allows entry into the 4-*O*-alkyl-D-glucopyranoses and D-glucuronic acid, and the oxidation methodology should also be suitable for the synthesis of uronosyl-

containing oligosaccharides.

5.4 Experimental

General.----Melting points were uncorrected and optical rotations were obtained at ambient temperatures. Evaporations were performed under reduced pressure at temperatures not exceeding 42°C (unless otherwise stated). All reactions were performed under an atmosphere of dry nitrogen. NMR spectra were recorded on a 400 MHz instrument operated at 27 °C. Chemical shifts (ppm) are relative to the central solvent peak of acetone-d_6 (^{13}C, 29.8 ppm ^{1}H, 2.04 ppm). Assignments are based on standard one- (^{1}H, ^{13}C, DEPT) and two-dimensional (homo- and hetero-nuclear) NMR experiments. Thin-layer chromatography was performed with Alugram Sil-G/UV_{254} plates (Macherey-Nagel) with visualization either with UV-light or by charring (5% H_2SO_4 in 95% EtOH). Column chromatography was with Silica Gel 60 (230-400 mesh, Whatman) using a standard flash chromatography apparatus (Ace Glass).

Methyl 2,3,6-tri-O-benzoyl-α-D-glucopyranoside (**2**). A mixture of methyl α-D-glucopyranoside (**1**) (1.00 g, 5.15 mmol), bis(tributyltin) oxide (4.61 g, 7.73 mmol) and toluene (50 mL) was placed in an oil-bath (140 °C) and refluxed overnight with a Dean-Stark trap (side arm prefilled with toluene). Subsequently, the side-arm was emptied and an additional amount of toluene was distilled over (ca. 25 mL). The mixture was then cooled under nitrogen atmosphere to about 22 °C, and a solution of benzoyl chloride (3.62 g, 25.75 mmol) in toluene (5 mL) was added dropwise with stirring over a five minute duration. The reaction was continued at 20-25 °C for five hours, at which time the mixture was concentrated under reduced pressure at 50 °C. Purification by silica gel chromatography (toluene, 200 mL then toluene:ethyl acetate, 10:1) gave methyl 2,3,6-tri-

O-benzoyl-α-D-glucopyranoside (**2**) (2.35 g, 4.64 mmol, 90.1%). Crystals from ethyl ether-hexane: mp 129-130 °C, $[\alpha]_D$ +147.8° (*c*=0.50, $CHCl_3$); lit.[8] mp 127-129 °C, $[\alpha]_D$ +149.4° (*c*=0.49, $CHCl_3$). NMR (acetone-d_6): δ_H (consistent with reported data[7]); δ_C: 55.44 (1-OCH_3), 64.45 (C-6), 69.57 (C-4), 70.82 (C-5), 73.02 (C-2), 74.12 (C-3), 97.85 (C-1), 166.19, 166.48, 166.62 (C=O)

*Benzyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside* (**6**). Compound **5** was tribenzoylated, as described for methyl α-D-glucopyranoside (**1**), to give **6** in 83% yield which could be crystallized from ethyl ether-hexane: mp 100-101°C, $[\alpha]_D$+144.1° (*c*=0.50, $CHCl_3$); NMR (acetone-d_6): δ_H 4.21 (1H, t, $J_{4,5}$, $J_{3,4}$ 9.6 Hz, H-4), 4.35 (1H, m, H-5), 4.66 (1H, d, J_{gem} 12.4 Hz, CH_2Ph), 4.68 (1H, dd, $J_{5,6b}$ 5.3 Hz, $J_{6a,6b}$ 11.9 Hz, H-6b), 4.77 (1H, dd, $J_{5,6a}$ 2.1 Hz, $J_{6a,6b}$ 11.9 Hz, H-6a), 4.91 (1H, d, J_{gem} 12.2 Hz, CH_2Ph), 5.28 (1H, dd, $J_{1,2}$ 3.7, $J_{2,3}$ 10.2 Hz, H-2), 5.39 (1H, d, $J_{1,2}$ 3.7 Hz, H-1), 6.00 (1H, dd, $J_{3,4}$ 9.2, $J_{2,3}$ 10.2 Hz, H-3); δ_C: 64.36 (Bn), 69.51 (C-4), 69.99 (C-6), 71.20 (C-5), 72.89 (C-2), 74.12 (C-3), 96.08 (C-1), 166.11, 166.43, 166.61 (C=O).

Anal. Calcd. for $C_{34}H_{30}O_9$: C, 70.09; H, 5.19. Found: C, 69.97; H, 5.26.

*Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-methyl-α-D-glucopyranoside* (**3**). Compound (**3**) was prepared by two different routes. For small scale reactions, methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**2**) was methylated with methyl triflate and 2, 6-di-*tert*-butyl-4-methylpyridine in dichloromethane[9] for three days, which gave **3** as a foam (92% yield). Large-scale reactions were based on the Kuhn methylation procedure.[10,11] Silver oxide (7.73 g, 33.37 mmol) was added to a solution of **2** (3.38 g, 6.67 mmol) and iodomethane (4.74 g, 33.37 mmol) in DMF (25 mL). The reaction mixture was stirred in the dark. When TLC ($CHCl_3$-EtOAc: 19/1) indicated that **2** had almost disappeared, the reaction was diluted with chloroform. The reaction mixture was filtered and washed with chloroform. The filtrate was washed with water four times and processed in standard fashion to afford

a syrup which was purified by silica gel chromatography ($CHCl_3$-EtOAc: 19/1) to give **3** (2.78 g, 80%). Compound **3** has been reported in the literature previously[15], but without physical data. $[\alpha]_D$ +132.1° (*c*=1.0, $CHCl_3$); NMR (acetone-d_6): δ_H 3.45 (3H, s, 1-OCH_3), 3.50 (3H, s, 4-OCH_3), 3.90 (1H, t, $J_{4,5}$, $J_{3,4}$ 9.7 Hz, H-4), 4.13 (1H, m, H-5), 4.59 (1H, dd, $J_{5,6b}$ 5.1 Hz, $J_{6a,6b}$ 12.0 Hz, H-6b), 4.71 (1H, dd, $J_{5,6a}$ 2.3 Hz, $J_{6a,6b}$ 12.1 Hz, H-6a), 5.15 (1H, d, $J_{1,2}$ 3.7 Hz, H-1), 5.19 (1H, dd, $J_{1,2}$ 3.6, $J_{2,3}$ 10.2 Hz, H-2), 5.94 (1H, dd, $J_{3,4}$ 9.2, $J_{2,3}$ 10.2 Hz, H-3); δ_C: 55.52 (1-OCH_3), 60.66 (4-OCH_3), 64.08 (C-6), 69.66 (C-5), 73.00 (C-2), 73.54 (C-3), 78.91 (C-4), 97.72 (C-1), 166.16 , 166.23, 166.52 (C=O).

Anal. Calcd. for $C_{29}H_{28}O_9$: C, 66.92; H, 5.42. Found: C, 66.96; H, 5.16.

Methyl 4-O-methyl-α-D-glucopyranoside (**4**). The solution of compound (**3**) (1.00 g, 1.92 mmol), $NaOCH_3$ (30 mg) and methanol (30 ml) was stirred at room temperature. When TLC ($CHCl_3$-MeOH, 6:1) showed that the reaction was complete, the mixture was neutralized with ion-exchange resin (H^+ form), filtered, and the filtrate was concentrated to give a syrup. The syrup was dissolved in water and evaporated to a syrup, and this operation was repeated three times. The syrup was dried under vacuum to give **4** quantitatively, which could be crystallized from ethyl acetate: mp 95-96 °C, $[\alpha]_D$ +158° (c=1.21, water); lit.[16] mp 94-95 °C, $[\alpha]_D$ +165° (c=1.2, water); lit.[17] mp 97-98 °C, $[\alpha]_D$ +167° (c=1.17, water). NMR (acetone-d_6/D_2O, 9:1): δ_H 3.06 (1H, dd, $J_{3,4}$ 8.9, $J_{4,5}$ 10.0 Hz, H-4), 3.30 (3H, s, 1-OCH_3), 3.37 (1H, dd, $J_{1,2}$ 3.7, $J_{2,3}$ 9.7 Hz, H-2), 3.43 (1H, m, H-5), 3.48 (3H, s, 4-OCH_3), 3.61 (1H, dd, $J_{5,6b}$ 4.7 Hz, $J_{6a,6b}$ 11.9 Hz, H-6b), 3.68 (1H, t, $J_{3,4}$, $J_{2,3}$ 9.2 Hz, H-3), 3.70 (1H, dd, $J_{5,6a}$ 2.3 Hz, $J_{6a,6b}$ 11.9 Hz, H-6a), 4.61 (1H, d, $J_{1,2}$ 3.7 Hz, H-1); δ_C: 55.21 (1-OCH_3), 60.30 (4-OCH_3), 60.90 (C-6), 71.18 (C-5), 71.97 (C-2), 73.53 (C-3), 79.75 (C-4), 99.68 (C-1).

Compound (**4**) can also be obtained from **2** without the purification of **3**. According to the

above procedures (silver oxide/iodomethane) and debenzoylation, crude (**4**) was obtained as a syrup which was purified with silica gel chromatography ($CHCl_3$-MeOH, 6:1) to give **4** (78% yield based on **2**).

Methyl 4-O-methyl -α-D-glucopyranosiduronic acid (**7**). The solution of compound (**4**) (1.00 g, 4.80 mmol), TEMPO (7.5 mg, 0.048 mmol), NaBr (0.25 g, 2.4 mmol) and de-ionized water (15 ml) was cooled to 0 °C in an ice-water bath. Sodium hypochlorite (5% solution, 0.79 g, 10.56 mmol, 14.3 mL) was added dropwise to the above reaction mixture. The pH value was kept at 10-11 by the dropwise addition of 0.5 N NaOH. TLC ($CHCl_3$-MeOH, 6:1) showed that the starting material (**4**) disappeared in 30 min. After a reaction time of 60 min, methanol (10 mL) was added to quench the reaction, and the mixture was evaporated to a solid which was extracted with methanol. The methanol extract was evaporated to a solid which was purified by silica gel ($CHCl_3$-MeOH-HOAc, 3/1/0.25) to give methyl 4-*O*-methyl α-D-glucopyranosiduronic acid (**7**) in 90% yield: $[\alpha]_D$ +129.3° (*c*=1.27,water) lit.[3] $[\alpha]_D$ +47° (*c*=1.02, water) NMR (acetone-d_6/D_2O,9/1): δ_H 3.26 (1H, dd, $J_{3,4}$ 9.0, $J_{4,5}$ 9.9 Hz, H-4), 3.32 (3H, s, 1-OCH_3), 3.42 (3H, s, 4-OCH_3), 3.45 (1H, dd, $J_{1,2}$ 3.8, $J_{2,3}$ 9.7 Hz, H-2), 3.67 (1H, t, $J_{3,4}$, $J_{2,3}$ 9.3 Hz, H-3), 3.89 (1H, d, $J_{4,5}$ 10.1 Hz, H-5), 4.67 (1H, d, $J_{1,2}$ 3.8 Hz, H-1); δ_C 55.65 (1-OCH_3), 60.35 (4-OCH_3), 70.60 (C-5), 72.15 (C-2), 73.56 (C-3), 81.83 (C-4), 100.69 (C-1), 171.89 (C-6), .

5.5 References

1. E. Sjöström, *Wood Chemistry, Fundamental and Applications*, 2nd Edition; Academic Press, Inc. 1993.

2. T. E. Timell, *Adv. Carbohydr. Chem.*, 19 (1964) 247-320.

3. J. Y. Lee, A. Ishizu, S. Hosoya and J. Nakano, *Cellul. Chem. Technol.*, 13 (1979) 739-745.

4. A. Wacek, F. Leilinger and P. Hochbahn, *Monatsh chem.*, 90 (1959) 562.

5. P. Kovác, *Carbohydr. Res.*, 31(1973) 323-330.

6. W. D. S. Bowering and T. E. Timell, *Can. J. Chem.*, 38 (1962) 311.

7. P. A. J. Gorin, *Can. J. Chem.*, 35 (1957) 595-598.

8. T. Ogawa and M. Matsui, *Tetrahedron*, 37(1981) 2363-2369.

9. J. Arnarp and J. Lonngren, *Acta Chem. Scand. B,* 32 (1978) 465-467.

10. R. Kuhn, H. Trischmann and I. Löw, Angew. Chem., 67 (1955) 32.

11. P. Kovác, J. Hirsch and V. Kovácik, *Carbohydr. Res.*, 58 (1977) 327-336.

12. J. Fabre, D. Betbeder, F. Paul and P. Monsan, *Syn. Comm.*, 23 (1993) 1357-1360.

13. N. J. Davis and S. L. Flitsch, *Tetrahedron Lett.*, 34 (1993) 1181-1184.

14. A. E. J. deNooy, A. C. Besemer and H. *van* Bekkum, *Recl. Trav. Chim. Pays-Bas* , 113 (1994) 165-166.

15. T. Aoyama and T. Shioiri, *Tetrahedron Lett.*, 31 (1990) 5507-5508.

16. R. L. Whistler, E. G. Linke and S. J. Kazeniac, *J. Am. Chem. Soc.*, 78 (1956) 4704-4709.

17. J. W. Van Cleve and C. R. Russell, *Carbohydr. Res.*, 25 (1972) 465-473.

Chapter 6

Synthesis and Rearrangement Reactions of Ester–Linked Lignin–Carbohydrate Model Compounds

6.1 Abstract

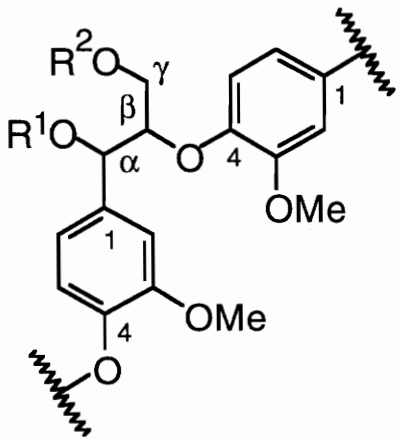
A series of ester-linked lignin–carbohydrate model compounds has been synthesized, which represent possible ester linkages of lignin to hemicellulose in wood through the carboxylic acid group of 4-*O*-methyl-α-D-glucopyranosiduronic acid moieties. The *threo* and *erythro* isomers of these compounds were prepared through an appropriate combination of protective and stereoselective transformations. Spectroscopic characterization of these esters revealed that the uronosyl group migrated between the primary (γ) and benzylic (α) positions of the lignin side chain in both acidic and neutral conditions. It has been determined that the migration equilibrium favors γ- over α-esters, with the reaction possibly proceeding through a six-membered ring intermediate. That uronosyl migrations occur favoring the γ-position suggests that the classical view of lignin-xylan ester linkages in wood needs to be reevaluated.

6.2 Introduction

Lignin and hemicellulose are theorized to be intimately associated with one another through covalent and non-covalent interactions (Fengel and Wegener, 1989). One type of covalent linkage thought to be present is an ester linkage between a xylan uronic acid moiety and the lignin polymer. Since the principal hemicellulose of temperate zone hardwood is *O*-acetyl-(4-*O*-methylglucurono)xylan (Timell, 1967), for such a linkage to occur the uronosyl

moiety of this polymer would be esterified to a lignin hydroxyl. Indeed, evidence has been reported for the existence of the ester linkages between lignin and 4-*O*-methyl-α-D-glucopyranosiduronic acid in lignin-carbohydrate complexes (LCC) isolated from hardwoods (Takahashi and Koshijima, 1988; Watanabe and Koshijima, 1988; Imamura *et al.*, 1994).

Results from these investigations support ester linkages between lignin and hemicellulose which form in accordance with the classical hypothesis of lignin biosynthesis (Freudenburg, 1968). In this scenario, lignin quinone methide intermediates undergo a nucleophilic attack by the carboxylic acid group of a uronic acid, affording a benzylic ester, or the so called α-ester (Structure A). In contrast to this result, it has been reported that maize rind lignin contains substantial amounts of *p*-coumaric acid esterified exclusively at the primary position of the lignin propyl side chain (γ-ester; Structure B) (Ralph *et al.*, 1994). This linkage cannot be explained by a quinone methide mechanism, implying a distinct biologically-controlled pathway may be responsible. If such a process exists in maize, it is also possible that lignin-hemicellulose ester linkages are under some level of enzymatic control, affording linkages such as Structure B.



Structure	R^1	R^2
A	acyl	H
B	H	acyl
C	H	H

In addition to this regiochemical issue, stereochemical questions pertaining to lignin-

polysaccharide structure have yet to even be considered. The most abundant lignin structural unit, the β-*O*-4 (Structure C), has two chiral centers in the propyl side chain, and hence can exist as a pair of diastereomers: *erythro* and *threo*. It has been reported that almost equal amounts of *erythro* and *threo* isomers exist in softwood spruce lignin, whereas *erythro* isomers are predominant in hardwood lignin (Lundquist, 1980; Nimz *et al.*, 1984; Hauteville *et al.*, 1986). It has been reported that various stereoselectivities were observed in the reaction between several carboxylic acids and a β-*O*-4 quinone methide (Sipilä and Brunow, 1991; Ralph and Young, 1983; Nakatsubo *et al.*, 1976). Whether or not an α-ester linkage between the naturally abundant 4-*O*-methyl-α-D-glucopyranosiduronic acid moiety of hardwood xylans and the lignin β-*O*-4 α-position has a stereochemical preference is not known.

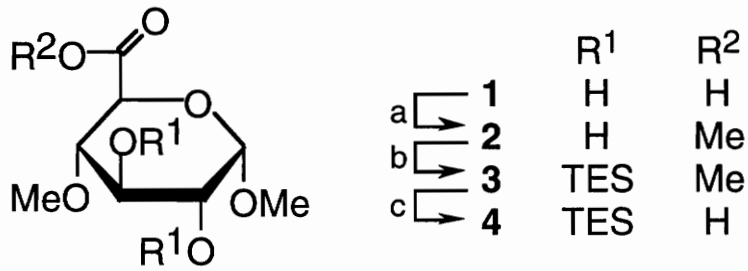
In efforts to elucidate these regiochemical and stereochemical questions, we have prepared several α- and γ- ester model compounds between methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid and the *threo* and *erythro* isomers of the lignin dimer, 1-(4-benzyloxy-3-methoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)propan-1-ol. During the course of this work, we observed migration of the 4-*O*-methyl-α-D-glucopyranosiduronate group between α- and γ-hydroxyl groups of the lignin dimer. The chemical aspects of the preparation of these compounds, their spectroscopic characterization and the kinetics of the migration are presented here, as well as the implications these results have on our understanding of lignin-xylan ester linkages in hardwoods.

6.3 Results and discussion

Synthetic Aspects and Spectroscopic Characterization. The carbohydrate portion of the lignin-carbohydrate esters was prepared as shown in Scheme I. Compound **1** (Li and Helm, 1995) was reacted with diazomethane in methanol to afford **2**.

Subsequently silylation with chlorotriethylsilane using imidazole as the base produced **3** (Berkowitz *et al.*, 1993). The target uronic acid **4** was prepared by saponification of **3** with 1N NaOH followed by neutralization with 0.05 N HCl. Crude **4** was submitted to the subsequent coupling reactions without further purification.

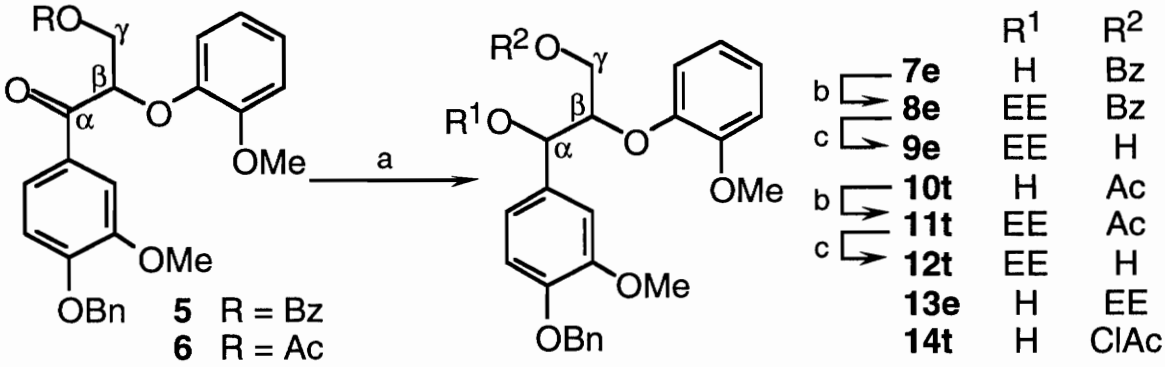
Scheme I. Synthesis of the Uronic Acid Portion of the LCC Models[a]



[a]a, CH_2N_2/MeOH; b, TES-Cl/imidazole; c, 1N NaOH (aq.) in MeOH.

The lignin portion was prepared in the following way (Scheme II). Compound **5** was reduced with zinc borohydride (Helm and Ralph, 1993) to afford the *erythro* isomers **7e**

Scheme II. Synthesis of the Lignin Portion of the LCC Models[a]



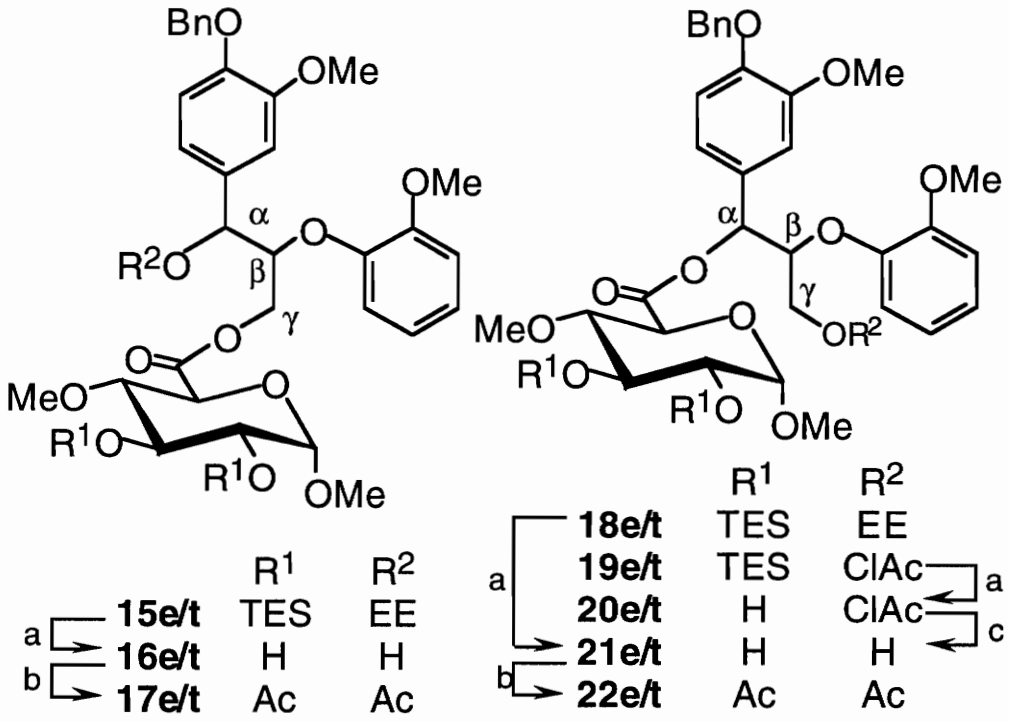
[a]a, $Zn(BH_4)_2$ or DIP-Cl; b, Ethyl vinyl ether/*p*-TsOH; c, NaOMe/MeOH.

in 91% yield. A benzoate protecting group was used instead of acetate to avoid acyl migration from the γ-hydroxyl to the α-hydroxyl during zinc borohydride reduction. Protection of the α-hydroxyl in **7e** with ethyl vinyl ether/*p*-toluenesufonic acid cleanly produced **8e**. Debenzoylation of **8e** with sodium methoxide in methanol afforded **9e** which was submitted to the coupling reaction without further purification. The *threo* isomers **10t** were prepared through the reduction of **6** with diisopinocampheylchloroborane (DIP-Cl) in THF (Helm and Li, 1995). As with the preparation of **8e** and **9e**, the ethoxyethylation of **10t** and subsequent deacetylation afforded **11t** and **12t**, respectively. Crude **12t** was submitted to the coupling step without further purification.

The lignin-carbohydrate esters were prepared by the coupling of lignin and carbohydrate moities as depicted in Scheme III. The DCC-mediated (DCC: 1,3-dicyclohexylcarbodiimide) esterification (Hassner and Alexanian, 1978) of **4** with **9e**, **12t**, **13e** (Helm and Ralph, 1993), and **14t** (Helm and Li, 1995) afforded **15e**, **15t**, **18e**, and **19t**, respectively. These compounds were deprotected (ethanolic 1% HCl) without characterization to afford **16e**, **16t**, **21e**, **20t**, respectively. The yield range of this two step process was 53–72%. Dechloroacetylation of **20t** with thiourea in 95% ethanol produced **21t** in 66% yield (Cook and Maichuk, 1970). Peracetylation of **16e**, **16t**, **21e**, **21t** with acetic anhydride, DMAP (4-dimethylaminopyridine) in CH_2Cl_2 afforded **17e**, **17t**, **22e**, **22t**, respectively in 92–95% yield.

Selected ^{1}H NMR data for the final products are shown in Table I. Placement of an optically active unit (the uronic acid) on a pair of enantiomers (either the *threo* or *erythro* isomers) affords a mixture of diastereomers. Thus the NMR spectra of the products show "peak doubling" due to the two diastereomers present. When extraction of spectroscopic data for each diastereomer was possible, the data are listed separately in Table I.

Scheme III. Preparation of the LCC Models[a]



[a]a,Ethanolic 1% HCl; b, Ac_2O/DMAP; c, Thiourea/95% EtOH, 70°C.

Assignment of the signals to individual diastereomers was not possible. For α-esters, the coupling constants ($J_{\alpha,\beta}$) of *threo* isomers are larger than those of *erythro* isomers, and this trend was also observed in the peracetylated products. The chemical shifts of γ^1-H and γ^2-H (with γ^2-H referring to the downfield γ-proton) in *erythro* isomers are much closer than those in *threo* isomers. The resonance signals of γ^1-H and γ^2-H in **16e**, **21e** and **17e** were completely overlapped prohibiting first-order analyses of coupling constants. The resonance signals of H-1 and β-protons also overlapped in the *erythro* isomers, whereas they were separated in the *threo* isomers. The $J_{\gamma 1,\beta}$ values are typically larger than $J_{\gamma 2,\beta}$ values in *threo* isomers, while the opposite is true of the *erythro* isomers, *i.e.* $J_{\gamma 1,\beta}<J_{\gamma 2,\beta}$. The chemical shifts of β-protons in the *threo* isomers are upfield relative

to their *erythro* counterparts. The α-proton chemical shifts of *threo* isomers are slightly downfield relative to the *erythro* isomers in the α-esters.

The above information can be used to differentiate *threo* and *erythro* isomers. The unacetylated α-esters have characteristic peaks (α-protons) at ca. 6.0 ppm, and no peaks between 4.1 and 4.5 ppm, whereas, the γ-esters have characteristic peaks (γ-protons) at 4.1-4.5 ppm and no peaks at ca. 6.0 ppm. Such information can aid in the identification of α and γ-esters in native LCC complexes, but it is imperative that NMR data of unacetylated material be obtained.

The selected ^{13}C NMR data are shown in Table II. As a general rule, the chemical shifts of the α-carbon in *threo* isomers are downfield from their *erythro* counterparts. Differentiation of α-esters and γ-esters can easily be achieved by the downfield shifts exhibited by the binding carbon of glucopyranosiduronate. For instance, the chemical shifts of α-carbons change from 72.83–72.92 ppm in γ-esters to 75.80–76.64 ppm in the α-esters; the chemical shifts of γ-carbons change from 64.88–65.09 ppm in γ-esters to 60.80–60.87 ppm in α-esters. This effect is lost upon peracetylation, which also increases the chemical shifts of the C-1 carbon (ca. 3 ppm) as well as all other ring carbons (ca. 1–2 ppm) except C-5 in the uronate moiety. It is also interesting to note that the C-4 signals in each pair of the isomers are more significantly different than all other carbon signals in the α-esters (**21e**, **22e**, **21t**, **22t**) but not in the γ-esters.

Uronosyl migration. During the deprotection of **15e** and **15t**, it was observed that the uronate group would migrate from the γ-position to the α-position if the reaction time was sufficiently long (>30 min.). The same deprotection procedure applied to **18e** afforded a mixture of α-ester **21e** and γ-ester **16e**. If **18e** was stirred in ethanolic 1% HCl for an

Table 1. Selected ^{1}H NMR data for α and γ-esters and their corresponding peracetates[a]

	γ-esters		α-esters		γ-esters-OAc		α-esters-OAc	
	16e	**16t**	**21e**	**21t**	**17e**	**17t**	**22e**	**22t**
α ($J_{\alpha,\beta}$)	4.96 (5.2)	4.96 (5.0)	6.06 (5.0)	6.11 (6.0)	6.05 (5.2)	6.11 (6.1)	6.14 (4.6)	6.14 (6.9)
α ($J_{\alpha,\beta}$)	4.96 (5.0)	4.97 (5.0)	6.06 (4.9)	6.13 (5.8)	6.06 (5.2)	6.13 (6.0)	6.14	6.16 (7.0)
β	4.64[b]	4.60	4.64[b]	4.60	4.90[b]	4.84[b]	4.90[b]	4.88
γ1 ($J_{\gamma1,\beta}$)	4.43[c]	4.04 (6.0)	3.66[d]	3.47 (5.0)	4.43[c]	4.06 (5.4)	4.25 (4.0)	4.00 (5.3)
γ1 ($J_{\gamma1,\beta}$)	4.43[c]	4.09 (6.3)	3.66[d]	3.50 (5.1)	4.43[c]	4.11 (5.5)	4.18 (4.3)	4.03 (5.4)
γ2 ($J_{\gamma2,\beta}$)	4.43[c]	4.37 (3.3)	3.76[e]	3.67[f]	4.43[c]	4.39 (3.3)	4.25 (6.1)	4.23 (3.9)
γ2 ($J_{\gamma2,\beta}$)	4.43[c]	4.41 (3.4)	3.76[e]	3.67[f]	4.43[c]	4.43 (3.4)	4.29 (6.2)	4.25 (4.0)
H-1 ($J_{1,2}$)	4.62[b] (3.7)	4.65 (3.7)	4.64[b]	4.62 (3.7)	4.90[b]	4.92[b] (3.4)	4.90[b]	4.87 (3.7)
H-1 ($J_{1,2}$)	4.63 (3.7)	4.66 (3.7)	4.64[b]	4.64 (3.5)	4.90[b]	4.93 (3.5)	4.90[b]	4.90 (3.5)
H-2 ($J_{2,3}$)	3.39[g]	3.43 (10.2)	3.39 (9.7)	3.40	4.74 (10.3)	4.74 (10.3)	4.65	4.73 (10.4)

—TABLE I, continued—

H-2 ($J_{2,3}$)	3.39[g]	3.44 (11.0)	3.43 (9.7)	3.40	4.76 (10.2)	4.78 (10.3)	4.70	4.76 (10.4)
H-3 ($J_{3,4}$)	3.66 (9.2)	3.68 (9.3)	3.66[d]	3.67[f]	5.37 (9.3)	5.37 (9.3)	5.38 (9.3)	5.36 (9.3)
H-3 ($J_{3,4}$)	3.66 (9.0)	3.68	3.66[d]	3.67[f]	5.37	5.38 (9.3)	5.39 (9.2)	5.37 (9.2)
H-4 ($J_{4,5}$)	3.23 (10.8)	3.26 (10.0)	3.22 (10.1)	3.18 (9.9)	3.54 (9.8)	3.56 (9.9)	3.57 (10.7)	3.55 (9.9)
H-4 ($J_{4,5}$)	3.24 (9.8)	3.31 (10.0)	3.22	3.27 (9.9)	3.55 (9.9)	3.57 (9.9)	3.62 (9.9)	3.57 (9.8)
H-5	3.88	3.90	3.95	3.94	4.01	4.04	4.12	4.05
H-5	3.88	3.92	3.96	3.95	4.03	4.08	4.14	4.09

[a]All spectra were recorded at 27 °C in acetone-d_6 (peracetates) or acetone-d_6 :D_2O (9:1; unacetylated). Due to the complex nature of these diastereomeric mixtures, complete resolution of all chemical shifts and coupling constants was not possible, nor was the assignment of the signals to the individual diastereomers. The discernible values are listed and coupling constants are to ± 0.2 Hz. [b]Signals of H-1 and β-H overlap. [c]Signals of γ^1-H and γ^2-H overlap. [d]Signals of H-3 and γ^1-H overlap. [e]Signals of γ^2-H and ArOMe overlap. [f]Signals of H-3 and γ^2-H overlap. [g]Signals of H-2 and 4-OMe overlap.

Table 2. Selected ^{13}C NMR Data for the α and γ-Esters and Their Corresponding Peracetates[a]

	γ-esters		α-esters		γ-esters-OAc		α-esters-OAc	
carbon	**16e**	**16t**	**21e**	**21t**	**17e**	**17t**	**22e**	**22t**
α	72.83	72.92	75.80 75.90	76.41 76.64	74.76 74.91	75.59 75.72	76.20 76.316	77.32 77.41
β	82.86 82.75	82.71 82.78	82.82 83.04	83.17 83.36	80.32 80.50	80.84 81.03	80.32 80.66	80.29 80.75
γ	64.88 65.01	64.89 65.09	60.80	60.87	64.75	65.21 65.26	63.25	63.53 63.65
C-1	101.14	101.01 101.03	101.04	100.96 101.01	98.18	98.24	98.22 98.28	98.12 98.20
C-2	72.71	72.40 72.42	72.43	72.41	71.45	71.45 71.48	71.40 71.45	71.41 71.46
C-3	74.14	73.82	73.87 73.98	73.86 74.06	71.87 71.92	71.90 71.95	71.90 72.03	71.82 72.14
C-4	81.73	81.57	81.41 81.82	81.34 82.07	79.56 79.67	79.60 79.69	79.49 79.89	79.41 80.08
C-5	70.07 70.99	70.77 70.88	70.95	70.92 71.01	70.75	70.68 70.88	70.90	70.76 70.99
C-6	170.13 170.16	170.11 170.06	168.99 169.21	169.26 169.69	168.95	168.77 168.89	167.97	168.10 168.41
$PhCH_2$	71.30	71.17	71.04 71.08	71.08	71.34	71.35	71.15 71.33	71.32
1-OCH_3	55.68	55.69	55.66	55.58 55.66	55.83	55.85 55.88	55.83	55.68 55.85
4-OCH_3	60.42	60.39	60.30	60.24 60.44	60.33	60.40	60.32 60.38	60.17 60.40
$ArOCH_3$	56.07 55.16	55.99 56.14	56.12	56.04 56.12 56.20	56.19 56.24	56.21 56.24	56.18 56.24	56.23

[a]The chemical shifts are in ppm and were measured at 27 °C in acetone-d_6 (peracetates) or acetone-d_6 : D_2O (9:1; unacetylated). Carbon designations are based on standard lignin and carbohydrate nomenclature, and are not assigned to individual diastereomers.

hour, the major compound was γ-ester **16e**, not α-ester **21e**. It was subsequently found that migration also would occur under neutral conditions. Compounds **16e**, **16t**, **21e** and **21t** were dissolved in NMR tubes with acetone-d_6:D_2O (9:1) and their ^1H NMR spectra were recorded over the course of several weeks. The ratio of γ-esters to α-esters was acquired by integration of α-protons of the α- and γ-esters, and the results are shown in Figure 1.

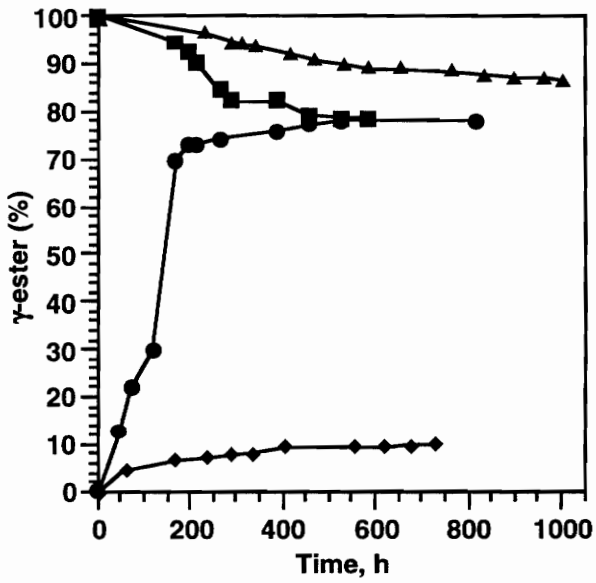


Figure 1. *Percent γ-Ester present in an NMR-Tube Experiment Versus Time.*
Starting Material: ■, *erythro* γ-ester (**20e**); ▲, *threo* γ-ester (**20e**); ●, *erythro* α-ester (**25e**); ◆, *threo* α-ester (**25e**).

The *erythro* isomers, whether starting from the α- or γ-esters, formed an equilibrium mixture in which the γ-ester predominated (80% γ-ester). However, in the time frame and solvent conditions investigated, it appears that the *threo* isomers are much less susceptible to the migration, as convergence to an equilibrium value starting from **16t** or **21t** has yet to occur. The difference in the rates of migration may be explained by examining the intermediates of the migration shown in Figure 2. Classical 1,3-diaxial interactions will occur only between the α-aryl group and the carbohydrate ring. If it is assumed that this interaction does not occur due to high energetics, the strongest interactions will be between the guaiacyl and phenyl rings. These moieties are gauche (*cis*) to each other in the *threo* intermediate, and would thus be of higher energy when compared to the *erythro* structure, where the two groups can assume an anti (*trans*) relationship. The diaxial orientation is

shown for the *erythro* isomers as this is the favored conformation for lignin model α,γ-phenylboronates and acetals (Ralph and Young, 1983).

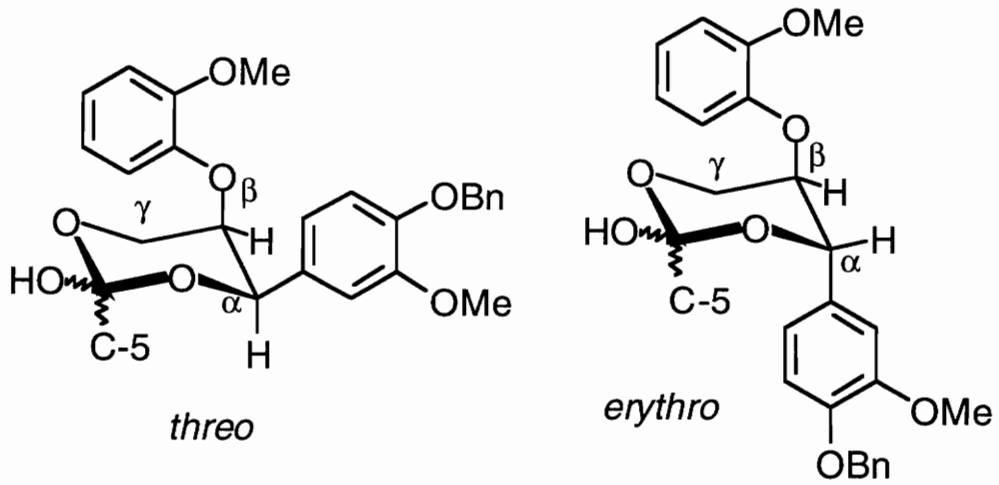


Figure 2. Intermediates for the uronosyl migrations. C-5 indicates the 5-position of the carbohydrate ring. Note the orientation of the aryl rings in the *threo* and *erythro* conformations.

This migration phenomenon has significant implications with respect to temperate zone hardwood LCC structures. If one were to assume that the LCC ester linkages are formed through the quinone methide intermediate, initial nucleophilic attack of the 4-*O*-methyl-α-D-glucopyranosiduronic acid moiety of glucuronoxylan would form an α-ester. The uronosyl group would then migrate from α-position to γ-position to form the equilibrium mixture. Although the time scale of the migration reaction may be long on the laboratory scale (Figure 1), it is very short with respect to the average lifespan of a temperate zone hardwood. The migration would be further aided by the acidic conditions present in the heartwood of hardwoods (brought about by cleavage of acetate groups). Therefore LCC isolation protocols as well as procedures utilizing DDQ methodology for quantitating LCC esters may need to be modified (Imamura *et al.*, 1994). The chemical and enzymatic susceptibility of the materials prepared in this study will be the subject of a separate

investigation.

In order to examine the chemistry of uronate/quinone methide coupling and the potential for migration, the reaction of methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid (**1**) and the quinone methide of 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propan-1,3-diol was performed. The quinone methide was prepared according to the procedure of Ralph and Young (1983), and was left exposed to methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid in chloroform/DMF for 4 days. The α-ester fraction was purified and ^{1}H NMR spectra were obtained for this sample in acetone-d_6:D_2O (9:1) over the course of several days. The initial spectrum revealed almost equal amounts of the *threo* and *erythro* isomers with a barely detectable amount of the γ-esters. Subsequent spectra showed the appearance of an increased amount of the γ-esters, approximately 8% after 100 h. The majority of the γ-esters were the *erythro* diastereomers, as would be expected from the results displayed in Figure 1.

6.4 Summary

Several α- and γ-ester LCC models and their peracetates have been prepared which accurately depict the theorized ester linkages between lignin and xylans in temperate zone hardwoods. The characterization of these compounds by NMR spectroscopy facilitated the differentiation between α-esters and γ-esters, as well as the *threo* and *erythro* isomers. During the course of these studies it was discovered that the 4-*O*-methyl-α-D-glucopyranosiduronate would migrate between the α- and γ-positions of the lignin side chain. The migration is an equilibrium which, in the case of *erythro* isomers, favored the γ-esters. The faster migration of *erythro* isomers relative to the *threo* isomers was attributed to differences in the cyclic intermediate of transesterification. It was also found

that an equal amount of *threo* and *erythro* α-esters were formed from the reaction of the β-*O*-4 quinone methide and methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid, and the transformation of these α-esters to γ-esters was also observed. Therefore, based on this study, it is proposed that if α-esters exist in hardwood cell walls (Imamura *et al.*, 1994), γ-esters will be present in higher concentrations. This potentially rules out enzyme involvement in the formation of uronosyl-lignin esters in wood.

6.5 Experimental

Moisture sensitive reactions were performed under an atmosphere of dry nitrogen, and evaporations were performed under reduced pressure at temperatures not exceeding 42 °C. Standard processing implies drying of the organic solvent with either Na_2SO_4 or $MgSO_4$, filtration, and evaporation of the solvent under diminished pressure. Trace solvent removal was accomplished under higher vacuum (25-75 mTorr). The NMR spectra were recorded at 400 MHz (Varian Unity 400) at 27 °C unless indicated otherwise, and chemical shifts (ppm) are relative to the central solvent peak of acetone-d_6 (^{13}C, 29.8 ppm; ^{1}H, 2.04 ppm). Thin-layer chromatography (TLC) was performed with AlugramSil-G/UV_{254} plates (Macherey-Nagel) with UV-light or by charring (5% H_2SO_4 in 95% EtOH). Column chromatography was with silica gel 60 (230-400 mesh, Whatman) using standard flash chromatography (Ace Glass).

Methyl (methyl 4-O-methyl-α-D-glucopyranosid)uronate (**2**). Diazomethane in Et_2O (Furniss *et al.*, 1989) was added to the solution of **1** (0.560 g, 2.52 mmol; Li and Helm, 1995) in methanol until the yellow color of diazomethane persisted, at which time the excess diazomethane was destroyed with acetic acid. The solution was evaporated to a

syrup and purified by silica gel chromatography ($CHCl_3$:EtOAc, 1:1) to afford **2** (0.554 g, 2.34 mmol) in 93% yield.

Methyl (methyl 2,3-di-O-triethylsilyl-4-O-methyl-α-D-glucopyranosid)uronate (**3**). Chlorotriethylsilane (0.80 mL, 4.78 mmol) was added dropwise to a solution of **2** (0.418 g, 1.77 mmol) and imidazole (0.602 g, 8.85 mmol) in DMF (4 mL). The solution was stirred at room temperature (1 h), and then poured into a separatory funnel which contained Et_2O:aq. satuated $NaHCO_3$ (30:30 mL). The aqueous phase was extracted with Et_2O (4x20 mL), and the combined ether phases were processed in a standard fashion to afford a thin syrup which was purified by silica gel chromatography (hexane:EtOAc, 15:1) to yield **3** (0.699 g, 1.50 mmol, 85%). NMR (acetone-d_6, ambient temperature): δ_H 0.67 (12H, q, J, 8.2 Hz, $Si(C\underline{H}_2CH_3)_3$), 0.98 (9H, t, J, 7.9 Hz, $Si(CH_2C\underline{H}_3)_3$), 0.99 (9H, t, J, 7.9 Hz, $Si(CH_2C\underline{H}_3)_3$), 3.19 (1H, dd, $J_{3,4}$ 8.7 Hz, $J_{4,5}$ 9.9 Hz, H-4), 3.35 (3H, s, 1-OCH_3), 3.36 (3H, s, 4-OCH_3), 3.54 (1H, dd, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 9.2 Hz, H-2), 3.76 (3H, s, Ac), 3.80 (1H, t, $J_{3,4}$, $J_{2,3}$ 8.9 Hz, H-3), 3.92 (1H, d, $J_{4,5}$ 9.9 Hz, H-5), 4.64 (1H, d, $J_{1,2}$ 3.5 Hz, H-1); δ_C 5.55, 5.81 ($Si(\underline{C}H_2CH_3)_3$), 7.04, 7.28 ($Si(CH_2\underline{C}H_3)_3$), 52.58 (6-$OCH_3$), 55.53 (1-$OCH_3$), 60.72 (4-$OCH_3$), 71.14 (C-5), 74.56 (C-2), 74.91 (C-3), 83.30 (C-4), 101.40 (C-1), 170.71 (C-6).

Methyl 2,3-di-O-triethylsilyl-4-O-methyl-α-D-glucopyranosiduronic acid (**4**). To a solution of **3** (0.097 g, 0.209 mmol) in methanol (2 mL) was added aq. NaOH (1N, 0.42 mL, 0.42 mmol). The mixture was stirred until the starting material had disappeared (monitored by TLC), at which time the solution was poured into a separatory funnel that contained a mixture of Et_2O (20 mL) and cold 0.05 N HCl (70 mL). The aqueous phase was extracted with Et_2O (3x20 mL), the combined ether phases was washed with water (1x) and processed in a standard fashion to afford **4** (0.088 g, 0.195 mmol, 94%). Crude

4 was submitted to the subsequent coupling reactions without further purification.

erythro *1-(4-benzyloxy-3-methoxyphenyl)-3-benzoyloxy-2-(2-methoxyphenoxy)propan-1-ol* (**7e**). Compound **5** (0.190 g, 0.37 mmol) was dissolved in EtOAc (4 mL) and cooled in ice-water bath. Ethereal $Zn(BH_4)_2$ (5 mL; Helm and Ralph, 1993) was added, and the reaction was monitored by TLC ($CHCl_3$:EtOAc; 19:1). When the reaction was complete, aq. NH_4Cl (1 mL) was added to quench the reductant. The solution was diluted with EtOAc, and washed with aq. NH_4Cl (3x). Standard processing and silica gel chromatography ($CHCl_3$:EtOAc, 19:1) afforded **7e** (0.173 g, 0.337 mmol, 91%) with >90% *erythro* selectivity.

erythro *1-(4-benzyloxy-3-methoxyphenyl)-3-benzoyloxy-1-(1-ethoxy)ethoxy-2-(2-methoxyphenoxy)propane* (**8e**). Compound **7e** was dissolved in CH_2Cl_2 (5 mL) and cooled in ice-water bath. Ethyl vinyl ether (0.16 mL, 1.67 mmol) was added and followed by adding trace amount of *p*-toluenesulfonic acid monohydrate (Helm and Ralph, 1993). The reaction was stirred for 1 h (TLC indicated that the reaction was complete in 10 min) and aq. $NaHCO_3$ was added to quench the reaction. The solution was diluted with EtOAc and washed with water (2x). Standard processing provided **8e** (0.192 g, 0.33 mmol, 99%) which was submitted to subsequent reactions without further purification.

erythro *1-(4-benzyloxy-3-methoxyphenyl)-1-(1-ethoxy)ethoxy-2-(2-methoxyphenoxy)-propan-3-ol* (**9e**). A solution of **8e** (0.192 g, 0.327 mmol) and sodium methoxide (10 mg) in methanol (10 mL) was stirred overnight at room temperature. The solution was poured into a separatory funnel which contained aq. NH_4Cl (30 mL) and $CHCl_3$ (30 mL). The aqueous phase was extracted with $CHCl_3$ (3x30), and the combined $CHCl_3$ layers was washed with water (1x), and processed in standard fashion. Trace methyl benzoate was removed by three additions and evaporations of water to the syrup. The syrup was

subsequently dried under high vacuum to give **9e** (0.155 g, 0.321 mmol, 98%)

threo *3-acetoxy-1-(4-benzyloxy-3-methoxyphenyl)-1-(1-ethoxy)ethoxy-2-(2-methoxy-phenoxy)propane* (**11t**). The protection of the α-hydroxyl in **10t** (0.207 g, 0.458 mmol) was accomplished as described for **8e**, to afford **11t** as a syrup (0.238 g, 0.454 mmol, 99%).

threo *1-(4-benzyloxy-3-methoxyphenyl)-1-(1-ethoxy)ethoxy-2-(2-methoxyphenoxy)propan-3-ol* (**12t**). Deacetylation of **11t** (0.210 g, 0.400 mmol), as described for **9e**, gave **12t** quantitatively.

erythro *3-(4-benzyloxy-3-methoxyphenyl)-3-hydroxyl-2-(2-methoxyphenoxy)propyl (methyl 4-O-methyl-α-D-glucopyranosid)uronate* (**16e**). Dicyclohexylcarbodiimide (DCC, 0.047 g, 0.228 mmol) was added to a solution of **4** (0.051 g, 0.113 mmol) in dry CH_2Cl_2 (distilled from CaH_2, 1 mL), followed successively by a solution of **9e** (0.0656 g, 0.136 mmol) in dry CH_2Cl_2 (1 mL) and DMAP (0.028 g, 0.229 mmol). The reaction was stirred overnight, subsequently filtered through Celite and washed with $CHCl_3$. The filtrate was processed to give crude **15e** which was dissolved in ethanolic 1% HCl (2 mL) and stirred for 0.5 h at room temperature. The reaction was terminated by adding satd. aq. $NaHCO_3$, and the neutralized reaction mixture was transferred to a separatory funnel, diluted with brine (20 mL) and extracted with $CHCl_3$ (4x30 mL). The combined $CHCl_3$ layers was processed in a standard fashion to give a syrup. The syrup was purified with preparative TLC (hexane:*i*-PrOH, 2:1) to afford **16e** (0.050 g, 0.081 mmol, 72% based on **4**).

erythro *3-acetoxy-3-(4-benzyloxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propyl*

*(methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosid)uronate* (**17e**). Acetic anhydride (0.0498 g, 0.046 mL, 0.488 mmol) and DMAP (0.0143 g, 0.117 mmol) were added a solution of **16e** (0.020 g, 0.0325 mmol) in dry CH_2Cl_2 (1 mL). The reaction was stirred overnight, diluted with $CHCl_3$, washed with cold 3% HCl (2x), and water (1x), and subsequently processed in standard fashion to afford **17e** (0.0229 g, 0.0309 mmol, 95%).

threo *3-(4-benzyloxy-3-methoxyphenyl)-3-hydroxyl-2-(2-methoxyphenoxy)propyl (methyl 4-*O*-methyl-α-D-glucopyranosid)uronate* (**16t**). The reaction of **4** (0.0926 g, 0.2054 mmol) with **12t** (0.1190 g, 0.2465 mmol), as described for **15e**, gave crude **15t** which was deprotected, as described for **16e**, to afford **16t** as a syrup (0.0858 g, 0.1397 mmol, 68% based on **4**).

threo *3-acetoxy-3-(4-benzyloxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propyl (methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosid)uronate* (**17t**). Compound **16t** (0.0289 g, 0.047 mmol) was peracetylated with acetic anhydride (0.072 g, 0.067 mL, 0.71 mmol), DMAP (0.021 g, 0.17 mmol), as described for **17e**, to afford **17t** (0.0327 g, 94%).

erythro *1-(4-benzyloxy-3-methoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)propyl (methyl 4-*O*-methyl-α-D-glucopyranosid)uronate* (**21e**). The reaction of **4** (0.0496 g, 0.1100 mmol) with **13e** (0.0637 g, 0.1320 mmol; Helm and Ralph, 1993), as described for **15e**, gave crude **18e** which was deprotected for 20 minutes, as described for **16e**, to afford **21e** (0.0282 g, 0.0459 mmol, 42%) and **16e** (0.0188 g, 0.0306 mmol, 28%). The overall yield of **21e** and **16e** was 70%.

erythro *3-acetoxy-1-(4-benzyloxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propyl (methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosid)uronate* (**22e**). Compound (**21e**) (0.0282 g, 0.0459 mmol) was peracetylated, as described for **17e**, to afford **22e** (0.0313, 0.0423 mmol, 92%).

threo *1-(4-benzyloxy-3-methoxyphenyl)-3-chloroacetoxy-2-(2-methoxyphenoxy)propyl (methyl 4-*O*-methyl-α-D-glucopyranosid)uronate* (**20t**). The reaction of **4** (0.0851 g, 0.1888 mmol) with **14t** (0.1104 g, 0.2266 mmol; Helm and Li, 1995), as described for **15e**, gave crude **19t** which was purified with silica gel chromatography (hexane:EtOAc, 2:1) to afford **19t** (0.1077 g, 0.117 mmol, 62%). The desilylation of **19t** (0.0910 g, 0.0989 mmol), was accomplished as described for **16e**, to afford crude **20t** which was subsequently purified by preparative TLC (EtOAc) to give **20t** (0.0581 g, 0.0841 mmol, 85%).

threo *1-(4-benzyloxy-3-methoxyphenyl)-3-hydroxyl-2-(2-methoxyphenoxy)propyl (methyl 4-*O*-methyl-α-D-glucopyranosid)uronate* (**21t**). The mixture of **20t** (0.0550 g, 0.0796 mmol) and thiourea (0.024 g, 0.318 mmol) in EtOH (95%, 2 mL) was stirred at 70 °C for 2 hours. The reaction mixture was diluted with water, then extracted with $CHCl_3$ (4x). The combined chloroform solution was processed in a standard fashion to give a syrup which was purified with preparative TLC (EtOAc) to give **21t** (0.0323 g, 0.0525 mmol, 66%).

threo *3-acetoxy-1-(4-benzyloxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propyl (methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosid)uronate* (**22t**). Compound **21t** (0.0252 g, 0.0410 mmol) was peracetylated, as described for **17e**, to afford **22e** (0.0279, 0.0423 mmol, 92%).

Quinone methide reactions. 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propan-1,3-diol (0.51 g, 0.158 mmol) was converted to the quinone methide in $CHCl_3$ (8 mL) as described by Ralph and Young (1983). The solution was dried and filtered (total volume ca. 15 mL $CHCl_3$), and a solution of **1** (0.090 g, 0.403 mmol) in DMF (1.5 mL) was added. The mixure was kept in the dark at room temperature for 4 days and subsequently evaporated under reduced pressure to remove the $CHCl_3$, and high vacuum to remove the DMF. The syrup was purified by preparative TLC ($CHCl_3$:MeOH, 6:1) to afford a *threo:erythro* mixture of 1-(4-hydroxy-3-methoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)propyl (methyl 4-*O*-methyl-α-D-glucopyranosid)uronate (0.022 g, 0.042 mmol, 26%). In a separate experiment the above procedure was followed except that 1,8-bis(dimethylamino)napthalene (Proton-Sponge, 2 mg, 0.0098 mmol) was added to the uronic acid/quinone methide solution and the reaction was stopped after two days. The yield of the α-ester was increased slightly (33%) and the purified product was a 1:1 mixture of *threo:erythro* isomers.

6.6 References

Berkowitz, D. B.; Eggen, M.; Shen, Q.; Sloss, D. G. Synthesis of (α,α-Difluoroalkyl)-phosphonates by Displacement of Primary Triflates. *J. Org. Chem.* **1993**. *58*, 6174-6176.

Cook, A. F.; Maichuk, D. T. Use of Chloroacetic Anhydride for the Protection of Nucleoside Hydroxyl Groups. *J. Org. Chem.* **1970**. *35*, 1940-1943.

Fengel, D.; Wegener, G. *Wood: Chemistry, Ultrastructure, Reactions.*, Walter de Gruyter, Berlin, **1989**, pp. 234-238.

Freudenberg, K. In *Constitution and Biosynthesis of Lignin*. Freudenberg, K. and Neish, A. C., Eds., Springer-Verlag, Berlin, **1968**, pp. 47-116.

Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. In *Vogel's Textbook of Practical Organic Chemistry*, 5th ed., John Wiley & Sons, New York. **1989**, pp. 430-433.

Hassner, A.; Alexanian, V. Direct Room Temperature Esterification of Carboxylic Acids. *Tetrahedron Lett.* **1978**. *46*, 4475-4478.

Hauteville, M.; Lundquist, K.; von Unge, S. NMR Studies of Lignins. ^{1}H NMR Spectroscopic Investigation of the Distribution of *erythro* and *threo* Forms of β-*O*-4 Structures in Lignins. *Acta Chem. Scand.* **1986**. B40, 331-35.

Helm, R. F.; Ralph, J. Stereospecificity for the Zinc Borohydride Reduction of α-Aryloxy-β-Hydroxy Ketones, *J. Wood Chem. Technol.* **1993**. *13*, 593-601.

Helm, R. F.; Li, K. Complete *threo*-Stereospecificity for the Preparation of β-*O*-4 Lignin Model Dimers. *Holzforschung* **1995**, 49, 533-536.

Imamura, T.; Watanabe, T.; Kuwahara, M.; Koshijima, T. Ester Linkages Between Lignin and Glucuronic Acid in Lignin-Carbohydrate Complexes From *Fagus crenata*. *Phytochemistry* **1994**. *37*, 1165-1173.

Li, K.; Helm, R. F. A Practical Synthesis of Methyl 4-*O*-Methyl-α-D-Glucopyranosiduronic Acid. *Carbohydr. Res.* **1995**, 273, 249-253.

Lundquist, K. NMR Studies of Lignins. 4. Investigation of Spruce Lignin by ^{1}H NMR Spectroscopy. *Acta Chem. Scand.* **1980**, *B34*, 21-26.

Nakatsubo, F.; Sato, K.; Higuchi, T. Enzymic Dehydrogenation of p-Coumaryl Alcohol. IV. Reactivity of the Quinonemethide. *Mokuzai Gakkaishi* **1976**, *22*, 29-33.

Nimz, H.H.; Tschirner, U.; Stahle, M.; Lehmann, R.; Schlosser, M. Carbon-13 NMR Spectra of Lignins, 10. Comparison of Structural Units in Spruce and Beech Lignin. *J. Wood Chem. Technol.* **1984**, *4*, 265-284.

Ralph, J.; Hatfield, R. D.; Quideau, S.; Helm, R. F.; Grabber, J. H.; Jung, H-J. G. Pathway of *p*-Coumaric Acid Incorporation into Maize Lignin as Revealed by NMR. *J. Am. Chem. Soc.* **1994**, *116*, 9448-9456.

Ralph, J.; Young, R. A. Stereochemical Aspects of Addition Reactions Involving Lignin Model Quinone Methides. *J. Wood Chem. Technol.* **1983**, *3*, 161-181.

Sipilä, J.; Brunow, G. On the Mechanism of Formation of Non-Cyclic Benzyl Ethers during Lignin Biosynthesis. Part 4. The Reactions of a β-*O*-4 type Quinone Methide with Carboxylic Acids in the Presence of Phenols. The Formation and Stability of Benzyl Esters between Lignin and Carbohydrates. *Holzforschung* **1991**, *45*, 9-14 (Suppl.).

Takahashi and Koshijima, T. Ester linkages between Lignin and Glucuronoxylan in a Lignin-Carbohydrate Complex from Beech (*Fagus crenata*) Wood. *Wood Sci. Technol.* **1988**, *22*, 231-241.

Timell, T. E. Recent Progress in the Chemistry of Wood Hemicelluloses, *Wood Sci. Technol.* **1967**, *1*, 45-70.

Watanabe, T.; Koshijima, T. Evidence for an Ester Linkage between Lignin and Glucuronic acid in Lignin Carbohydrate Complexs by DDQ Oxidation. *Agric. Biol. Chem.*, **1988**, 52, 2953-2955.

Chapter 7

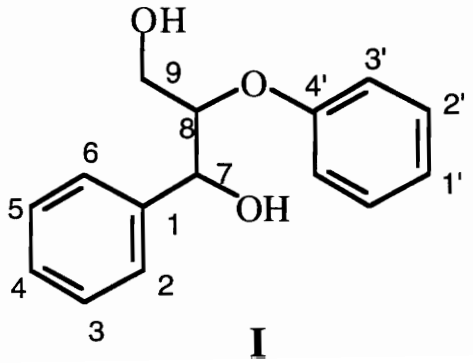
Approaches to Synthetic Neolignans

7.1 Abstract

Several synthetic approaches to neolignans with an 8-*O*-4' linkage are described. Although direct introduction of a chiral aryloxy ether bond at the 8-position of protected phenylpropane derivatives was not successful, an efficient method to synthesize a chiral aryloxy ether bond has been developed starting from a tartrate derivative.

7.2 Introduction

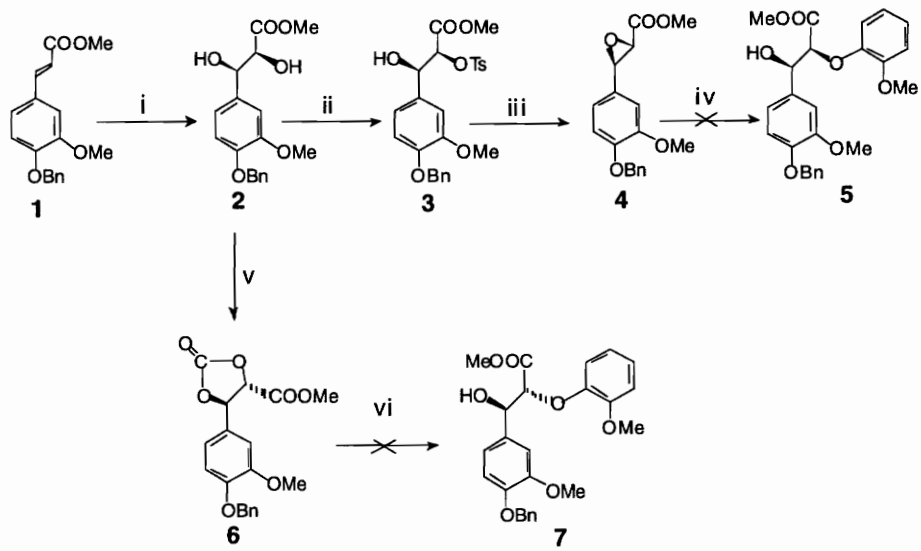
Neolignans are a group of natural products having very broad structural variation, and a large number of neolignans have been isolated and characterized in recent years.[1] One of the major classes of neolignans contains a chiral aryloxy 8-*O*-4' ether bond (**I**), and this type of neolignan is structurally analogous to the major interunit linkage of lignin. Whereas lignans and neolignans are optically active small molecules (typically dimers), lignin is an optically inactive polymer. The biosynthetic relationship among lignans, neolignans and lignin is still relatively unclear. Due to this current lack of understanding and the biological activity of numerous lignans and neolignans, we have begun a research program directed at developing asymmetric synthesis of several neolignans. In this communication our efforts to prepare 8-*O*-4' neolignans are described.



I

7.3 Results and discussion

Our first synthetic strategy was based on a Sharpless asymmetric dihydroxylation to prepare a chiral diol of a protected cinnamic acid derivative.[2] It was hoped that selective manipulation of the chiral diol would lead to a chiral aryloxy ether bond at the 8-position. Sharpless asymmetric dihydroxylation of **1** with AD-mix-β cleanly afforded optically active **2** in 68% yield (Scheme 1).[2] Selective tosylation of **2** with *p*-TsCl/Et_3N afforded

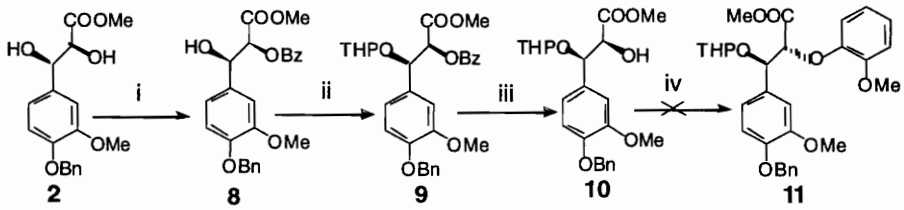


Scheme 1. *Reagents and conditions:* i, AD-mix-β; ii, *p*-TsCl/Et_3N; iii, K_2CO_3/DMF; iv, 2-MeOPhONa/dioxane; v, triphosgene/pyridine; vi, Guaiacol/Et_3N.

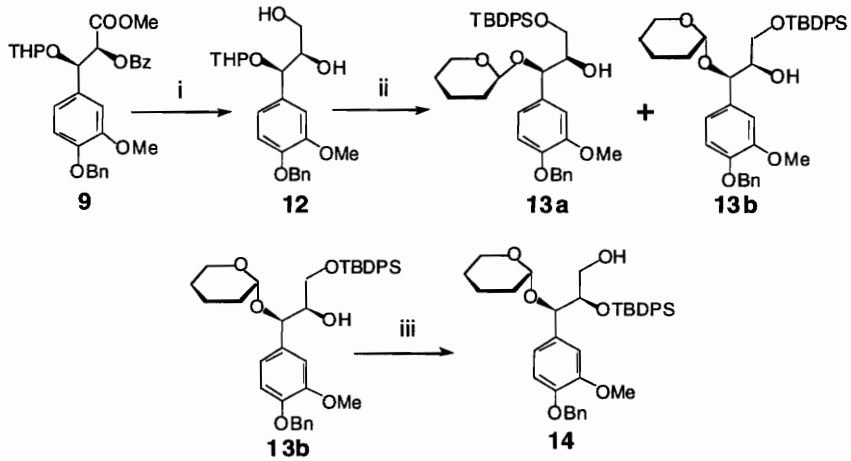
crystalline **3** in 90% yield,[3] and subsequent epoxidation of **3** with potassium carbonate in DMF provided **4** as a syrup in 84% yield.[3] Although ring-opening of an epoxide analogous to **4** with sodium 2-methoxyphenolate has been reported to occur exclusively at the 8-position,[4] the reaction of **4** with sodium 2-methoxyphenolate under various reaction conditions only provided an uncharacterized mixture of products. Another attempt to

synthesize 8-*O*-4' neolignans was inspired by a recent report in which a cyclic carbonate analogous to **6** was treated with thiophenol and triethylamine to afford a ring-opening product exclusively at the 8-position.[5] However, compound **6**, prepared from **2** with triphosgene/pyridine in 90% yield,[6] did not react with guaiacol using triethylamine or imidazole as a base.

In light of these setbacks, we next concentrated on the Mitsunobu reaction to synthesize the alkyl aryl ether (Scheme 2).[7] Selective benzoylation of the 8-hydroxyl in **2**, protection of



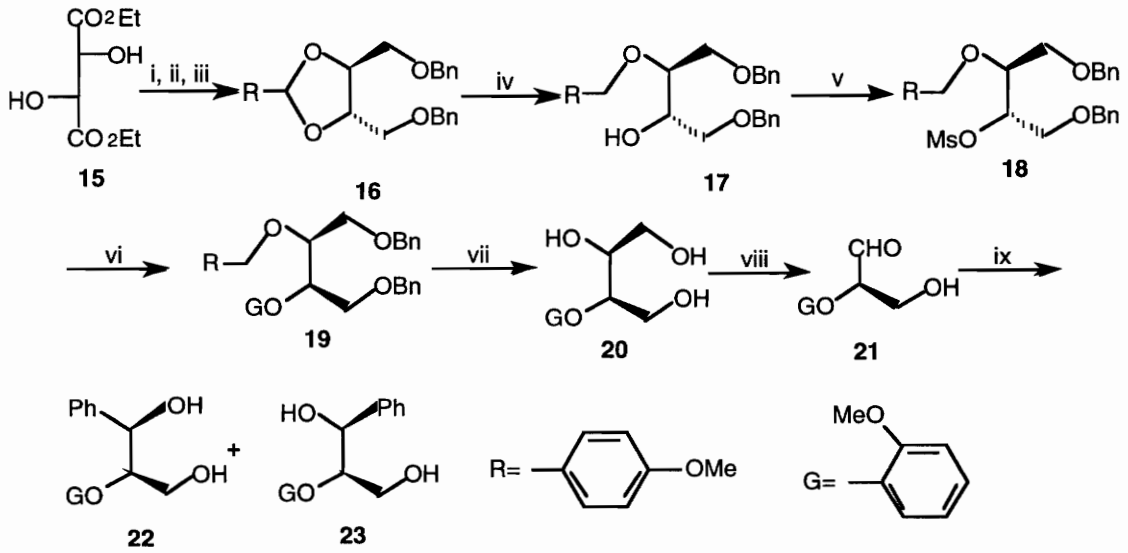
Scheme 2. *Reagents and conditions:* i, BzCl/Et_3N; ii, DHP/*p*-TsOH; iii, NaOMe/MeOH; iv, Guaiacol/DEAD/Ph_3P.



Scheme 3. *Reagents and conditions*: i, $LiAlH_4$/THF; ii, TBDPS-Cl/imidazole; iii, Guaiacol/DEAD/Ph_3P.

the 7-hydroxyl with THP, and subsequent debenzoylation afforded **10** in 79% overall yield. Unfortunately, the Mitsunobu reaction of **10** with guaiacol provided a mixture instead of the desired product (**11**). Another attempt to synthesize 8-O-4' neolignans is shown in Scheme 3. Compound **9** was reduced with lithium aluminum hydride to afford **12** in 83% yield. Selective protection of the primary hydroxyl group of **12** with TBDPS-Cl (*tert*-butyldiphenylsilyl chloride) and imidazole afforded **13a** and **13b** in 92% yield. The two diastereomers, **13a** and **13b**, were separated by preparative TLC. Submission of **13b** to the Mitsunobu reaction with guaiacol resulted in silyl migration; compound **14** was isolated in 62% yield.

Due to the difficulty associated with construction of a chiral aryloxy ether bond at the 8-position of the phenylpropanoid side chain, our attention turned to the initial synthesis of a



Scheme 4. *Reagents and conditions:* i, 4-methoxybenzaldehyde dimethyl acetal/ *p*-TsOH/DMF; ii, $NaBH_4$/EtOH; iii, BnBr/KOH/toluene; iv, DIBAL-H/toluene; v, MsCl/pyridine; vi, cesium 2-methoxyphenolate/18-crown-6; vii, H_2/Pd-C/ MeOH:HCOOH (9:1); viii, $Pb(OAc)_4$/benzene; ix, PhLi/THF.

chiral aryloxy ether bond, then introduction of an aromatic ring at the 7-position (Scheme 4). A three-step reaction sequence, namely the protection of diol as an acetal, reduction and benzylation, without purification of the intermediate compounds provided **16** from **15** in 71% yield.[8,9] The reductive ring-opening of **16** using diisobutylaluminum hydride (DIBAL-H) provided **17** in 97% yield.[10] Compound **17** was mesylated in pyridine to provide **18** in 98% yield. Compound **18** was completely converted to **19** by refluxing the mixture of **18**, cesium 2-methoxyphenolate and 18-crown-6 in benzene for 24 hrs. Hydrogenation of **19** with Pd/C in methanol-formic acid (9:1) provided **20** in 76% yield based on **18**. Diol cleavage of **20** with lead tetraacetate in dry benzene provided **21** which was submitted to next step without purification. The reaction of **21** with excess phenyl lithium at 0 °C provided equal amounts of two diastereomers, **22** and **23**, in 72% yield based on **20**. The separation of **22** and **23** was achieved by ion-exchange chromatography (QAE-Sephadex, A-25) using 0.06 M $K_2B_4O_7$ in acetone-water(1:4) as the eluent.[11] Compounds, **22** and **23**, have greater than 96% ee as determined by NMR of the bis-Mosher esters of **22** and **23**.

7.4 Conclusions

In conclusion, several synthetic routes to neolignans with 8-*O*-4' linkages have been investigated. Direct introduction of an aryloxy ether bond at the 8-position of a phenylpropane sidechain was not successful. As an alternative, an efficient method to synthesize a chiral aryloxy ether bond has been established, using cesium phenolate and 18-crown-6. This has led to the preparation of two optical neolignans of high enantiomeric excess. Through utilization of these protocols, the synthesis of several neolignans with 8-O-4' linkages and 1,4-benzodioxane structure is reported in Chapter 8.

7.5 Experimental

Organic solvents for silica gel chromatography were obtained from commercial sources and used without further purification. The reaction solvents were purified before use: methylene chloride, benzene and toluene were distilled from CaH_2, THF was distilled from Na, and pyridine was distilled from KOH. Moisture sensitive reactions were performed under an atmosphere of dry nitrogen. Evaporations were performed under reduced pressure at temperature not exceeding 42 °C. Standard processing implies that organic solvents were dried with Na_2SO_4 or $MgSO_4$, filtered and evaporated. Drying of products was accomplished under high vacuum (25-75 mTorr). Silica gel chromatography was performed using silica gel 60 (230-400 mesh, Whatman). Thin layer chromatography (TLC) was performed with Alugram Sil-G/Uv254 plates (Macherey-Nagel) with UV light or by charring (5% H_2SO_4 in 95% EtOH). Preparative TLC was performed on Silica-G/glass (20x20 cm, 1000 μm plate thickness, Whatman). NMR spectra were recorded at 400 MHz (Varian Unity 400) at ambient temperature with acetone-d_6 as solvent (unless indicated otherwise) and internal reference (central solvent peak, δ_H, 2.04 ppm; δ_C, 29.8 ppm) .

Methyl (2S,3R)-2,3-dihydroxy-3-(4-benzyloxy-3-methoxyphenyl)propionate (2). A mixture of AD-Mix-β (1.4 g) in *t*-BuOH (5 mL) and water (5 mL) was stirred at ambient temperature until two clear phases were generated. Methanesulfonamide (0.095 g, 1.00 mmol) was added, and stirred until two clear phases appeared. Methyl 4-benzylferulate (0.298 g, 1.00 mmol) was then added, and the reaction mixture was stirred until TLC (chloroform-EtOAc, 19:1) indicated that the starting material disappeared. Sodium bisulfite (1.5 g) was then added, and stirred for 0.5 hour. The reaction mixture was extracted with dichloromethane (5x20 mL), and the combined organic phase was subsequently washed with 2 N KOH (1x). Standard processing afforded a syrup which

was purified by silica gel chromatography (EtOAc-hexane, 2:1) to provide **2** (0.244 g, 73%) as a syrup. The crystallization from dichloromethane-cyclohexane provided crystalline **2** (0.227 g, 68%) as long needles. mp. $[\alpha]_D$ -2.13 (c 1.08 in $CHCl_3$). δ_H (acetone-d_6) 3.68 (3H, s, OCH_3); 3.82 (3H, s, OCH_3); 4.11 (1H, dd, $J_{OH,\ 2}$ 7.3, OH-2); 4.24 (1H, dd, $J_{2,\ OH}$ 7.0, $J_{2,\ 3}$ 3.5, H-2); 4.46 (1H, d, $J_{3,\ OH}$ 5.7, OH-3); 4.92 (1 H, dd, $J_{3,\ OH}$ 5.7, $J_{2,\ 3}$ 3.7, H-3); 5.09 (2 H, s, benzylic H), 6.90-7.50 (8 H, m, aromatic H); δ_C (acetone-d_6) 52.10 (OCH_3), 56.18 ($ArOCH_3$), 71.48 (benzylic C), 75.13 (C-3), 76.58 (C-2), 112.03, 114.72, 119.62, 128.41, 128.48, 129.16, 135.77, 138.73, 148.65 and 150.56 (aromatic C), 173.57 (C=O).

*(2S,3R)-methyl 3-(4-benzyloxy-3-methoxyphenyl)-3-hydroxy-2-(p-tolylsulfonyloxy)propionate (**3**).* A solution of **2** (0.146 g, 0.439 mmol) and triethylamine (0.067 g, 0.66 mmol) in dichloromethane (2.2 mL) was stirred and cooled in an ice-water bath. *p*-Tosyl chloride (0.086 g, 0.45 mmol) was added. The solution was stirred at the same temperature for 8 hrs, then evaporated, purified by silica gel chromatography (EtOAc-hexane, 1:1) to provide a syrup which crystallized from EtOAc-hexane to afford **3** (0.192 g, 90%). δ_H (acetone-d_6) 2.39 (3H, s, CH_3), 3.65 (3H, s, OCH_3); 3.69 (3H, s, OCH_3); 4.88 (1H, d, $J_{3,\ 2}$ 3.7, H-2); 4.95 (1H, d, $J_{3,\ OH}$ 6.1, OH); 5.09 (1H, dd, $J_{3,\ 2}$ 3.7, $J_{3,\ OH}$ 6.0, H-3); 5.11 (2 H, s, benzylic H), 6.77-7.54 (12 H, m, aromatic H); δ_C (acetone-d_6) 21.57 (CH_3), 52.58 (OCH_3), 55.76 ($ArOCH_3$), 71.22 (benzylic C), 73.81 (C-3), 82.8 (C-2), 110.82, 114.08, 128.37, 128.44, 128.54, 129.20, 130.36, 132.90, 133.82, 138.62, 145.64, 148.91 and 150.32 (aromatic C), 168.19 (C=O).

*(2R,3R)-methyl 3-(4-benzyloxy-3-methoxyphenyl)oxiranecarboxylate (**4**).* A mixture of **3** (1.06 g, 2.18 mmol), K_2CO_3 (0.90 g, 6.54 mmol), H_2O (0.2 mL, 10.9 mmol) and

DMF (11 mL) was stirred at ambient temperature until TLC showed that **3** disappeared. The reaction mixture was diluted with cold water, extracted with diethyl ether (5x). Standard processing provided a syrup which was purified by silica gel chromatography (hexane-EtOAc, 2:1) to afford **4** (0.62, 91%). δ_H (acetone-d_6) 3.55 (3H, s, OCH_3); 3.81 (3H, s, OCH_3); 3.88 (1H, d, $J_{3,2}$ 4.6, H-2); 4.25 (1H, d, $J_{3,2}$ 4.6, H-3); 5.09 (2 H, s, benzylic H), 6.94-7.49 (8 H, m, aromatic H); δ_C (acetone-d_6) 52.10 (OCH_3), 56.09 ($ArOCH_3$), 56.97, 57.84 (C-2, C-3), 71.38 (benzylic C), 111.37, 114.50, 120.25, 127.44, 128.49, 128.57, 129.17, 138.43, 149.52 and 150.47 (aromatic C), 167.69 (C=O).

*Cyclic carbonate **6**.* A solution of **2** (0.100 g, 0.300 mmol), pyridine (0.15 mL) in dichloromethane (1.5 mL) was stirred and cooled to -70 °C. A solution of triphosgene (0.045 g, 0.15 mmol) in dichloromethane was added. The solution was allowed to warm up to room temperature. After two hours, the reaction was quenched with aqueous NH_4Cl, diluted with dichloromethane and washed successively with cold 3% HCl, saturated aqueous $NaHCO_3$ and brine. Standard processing provided a syrup which was purified by preparative TLC (hexane-EtOAc, 2:1) to afford **6** (0.098 g, 90%). δ_H (acetone-d_6) 3.82 (3H, s, OCH_3); 3.86 (3H, s, OCH_3); 5.15 (2 H, s, benzylic H); 5.29(1H, d, $J_{3,2}$ 6.4, H-2); 5.81 (1H, d, $J_{3,2}$ 6.4, H-3); 7.03-7.51 (8 H, m, aromatic H); δ_C (acetone-d_6) 53.34 (OCH_3), 56.26 ($ArOCH_3$), 71.20 (benzylic C), 79.49, 81.04 (C-2, C-3), 111.11, 114.56, 120.35, 128.43, 128.64, 129.20, 138.08, 150.51 and 151.18 (aromatic C), 153.93, 167.69 (C=O).

*(2S,3R)-methyl 2-benzoyloxy-3-hydroxy-3-(4-benzyloxy-3-methoxyphenyl)propionate (**8**).* A solution of **2** (0.67 g, 2.02 mmol) in CH_2Cl_2 (10 mL) was stirred and cooled to -60 °C. Benzoyl chloride (0.29 g, 2.08 mmol) was slowly added. The solution was

stirred at -15 °C for about 2 hrs, then quenched with methanol, diluted with $CHCl_3$, and washed with brine (2x). Standard processing gave a syrup which was purified by silica gel chromatography ($CHCl_3$, then hexane-EtOAc, 2:1) to afford **8** (0.76 g, 87%). δ_H (acetone-d_6) 3.69 (3H, s, OCH_3); 3.78 (3H, s, OCH_3); 5.10 (2 H, s, benzylic H); 5.35(1H, d, $J_{3,2}$ 3.5, H-3); 5.43 (1H, d, $J_{3,2}$ 3.5, H-2); 6.95-8.11 (13 H, m, aromatic H); δ_C (acetone-d_6) 52.41 (OCH_3), 56.05 ($ArOCH_3$), 71.28 (benzylic C), 73.68 (C-3), 78.00 (C-2), 111.55, 114.49, 119.14, 128.42, 129.09, 129.36, 130.48, 134.27, 134.41, 148.77 and 150.54 (aromatic C), 166.11, 168.97 (C=O).

*(2S,3R)-methyl 2-benzoyloxy-3-(4-benzyloxy-3-methoxyphenyl)-3-(tetrahydropyranosyloxy)propionate (**9**).* To a solution of **8** (0.083, 0.19 mmol) in CH_2Cl_2 at -15 °C is added 2,3-dihydro-2H-pyran (0.17 mL, 1.9 mmol), and *p*-TsOH•H_2O (2 mg). After 2.5 hrs, saturated aqueous $NaHCO_3$ (0.5 mL) was added to quench the reaction. The reaction was then diluted with $CHCl_3$ and washed with brine (2x). Standard processing provided crude **9** which was submitted to next step without further purification.

*(2S,3R)-methyl 3-(4-benzyloxy-3-methoxyphenyl)-2-hydroxy-3-(tetrahydropyranosyloxy)propionate (**10**).* A solution of crude **9** (0.0935 g, 0.180 mmol), NaOMe (5 mg) and methanol (5 mL) was stirred at room temperature overnight. The reaction was then quenched with aqueous NH_4Cl (0.5 mL), diluted with $CHCl_3$, and washed with brine (2x). Standard processing afforded a syrup which was purified by preparative TLC (hexane-EtOAc, 2:1) to provide **10** (0.068 g, 91% based on **8**). δ_C (acetone-d_6) 19.36, 19.50, 26.10, 26.18, 31.11, 31.46 (CH_2), 51.97, 52.06 (OCH_3), 56.12, 56.13 ($ArOCH_3$), 61.69, 62.24 (O CH_2), 71.32, 71.36 (benzylic C), 75.97, 76.30, 78.22, 80.49 (C-3, C-2), 95.10, 99.88 (OCHO), 112.51, 112.79, 114.31, 114.37, 120.17, 121.00, 128.39, 128.42, 128.46, 128.48, 129.12, 129.14, 131.84,

133.82, 138.56, 138.61, 148.65, 149.09, 150.28 and 150.45 (aromatic C), 173.13, 173.26 (C=O).

*(2R,3R)-3-(4-benzyloxy-3-methoxyphenyl)-3-(tetrahydropyranosyloxy)-1,2-propanediol (**12**).* A solution of crude **9** (0.036 g, 0.068 mmol) in THF (1mL) is added to a flask containing 1.0 M lithium aluminum hydride in THF (0.41 mL) under an atmosphere of nitrogen. The solution was stirred at 60 °C, and after 3 hrs the mixture was quenched with EtOAc (5 mL), and filtered. The residue was thoroughly washed with EtOAc, and standard processing provided a syrup which was purified by preparative TLC (EtOAc-hexane, 2:1) to provide **12** (0.022 g, 83%). δ_C (acetone-d_6) 19.71, 20.11, 26.20, 26.24, 31.11, 31.46 (CH_2), 56.10 and 56.14 ($ArOCH_3$), 62.06, 62.69, 63.62, 63.72 (OCH_2), 71.37 and 71.42 (benzylic C), 76.36, 76.76, 78.81, 80.53 (C-3, C-2), 96.07 and 100.14 (OCHO), 112.40, 114.47, 120.22, 120.87, 128.43, 128.46, 128.48, 128.51, 129.13, 129.16, 133.16, 135.27, 138.59, 138.66, 148.52, 149.00, 150.39 and 150.68 (aromatic C).

*(1R,2R)-1-(4-benzyloxy-3-methoxyphenyl)-1-(tetrahydropyranosyloxy)-3-(tert-butyldiphenylsilyloxy)-2-propanol (**13a** and **13b**).* *tert*-Butyldiphenylsilyl chloride (0.183 g, 0.664 mmol) was added to a stirred solution of **12** (0.235 g, 0.604 mmol) and imidazole (0.062 g, 0.906 mmol) in DMF (7 mL) at room temperature, and the solution was stirred overnight. The solution was quenched by aqueous $NaHCO_3$, and extracted with ethyl acetate (4x). Standard processing provided a syrup which was purified by silica gel chromatography ($CHCl_3$, then hexane-EtOAc, 2:1) to afford **13a and 13b** (0.349, 92%). Compounds **13a** and **13b** were separated by preparative TLC (hexane-EtOAc, 2:1). Compound **13a**: δ_C (acetone-d_6) 19.73 (CH_2), 19.77 (Me_3CSi), 26.24 (CH_2), 37.19 (CH_3), 31.14 (CH_2), 56.12 ($ArOCH_3$), 62.09 (OCH_2), 65.67 ($SiOCH_2$), 71.44 (benzylic C), 76.58, 80.32 (C-1, C-2), 100.29 (OCHO), 112.55, 114.47, 120.33,

128.15, 128.48, 128.51, 128.52, 129.14, 130.33, 134.17, 134.39, 135.23, 136.24, 136.37, 138.68, 148.55 and 150.37 (aromatic C). Compound **13b**: δ_C (acetone-d_6) 19.81 (Me_3CSi), 19.92 (CH_2), 26.25 (CH_2), 27.18 (CH_3), 31.37 (CH_2), 56.09 ($ArOCH_3$), 62.43 (OCH_2), 65.73 ($SiOCH_2$), 71.39 (benzylic C), 76.49, 78.08 (C-1, C-2), 95.64 (OCHO), 112.54, 114.49, 121.05, 128.43, 128.46, 128.50, 129.17, 130.43, 133.17, 134.26, 134.54, 136.25, 136.42, 138.60, 149.06 and 150.66 (aromatic C).

*(2R,3R)-3-(4-benzyloxy-3-methoxyphenyl)-3-(tetrahydropyranosyloxy)-2-(tert-butyldiphenylsilyloxy)-1-propanol (**14**).* Diethyl azodicarboxylate (0.0229 g, 0.132 mmol) was added to a solution of Ph_3P (.0346 g, 0.132 mmol) in THF (0.5 mL) at -60 °C. The mixture was stirred for about 5 minutes at -60 °C. A solution of **13b** (0.0276 g, 0.0440 mmol) was added. The mixture was stirred for 5 minutes before guaiacol (0.0109 g, 0.0880 mmol) was added. The reaction was allowed to slowly warm to room temperature, stirred overnight, and evaporated. The residue was dissolved in EtOAc-hexane (3:7) and filtered. The filtrate was evaporated and purified by preparative TLC ($CHCl_3$-EtOAc, 19:1) to provide **14** (0.0171 g, 62%). δ_C (acetone-d_6) 20.00 (Me_3CSi), 20.54 (CH_2), 26.14 (CH_2), 27.42 (CH_3), 31.48 (CH_2), 56.01 ($ArOCH_3$), 63.28, 63.56 (OCH_2), 71.45 (benzylic C), 77.68, 78.29 (C-2, C-3), 96.93 (OCHO), 112.61, 114.62, 120.75, 128.28, 128.42, 128.50, 129.17, 130.35, 130.40, 133.15, 134.73, 135.30, 136.72, 138.68, 148.76 and 150.67 (aromatic C).

*(2S,3S)-1,4-Di-O-benzyl-2,3-(4-methoxybenzylidene)threitol (**16**).*[9-11] A solution of diethyl L-tartrate (5.35 g, 25.9 mmol), 4-methoxybenzaldehyde dimethyl acetal (7.09 g, 38.9 mmol), *p*-TsOH•H_2O (50 mg, 0.26 mmol) in DMF (60 mL) was placed in a rotavapor, and evaporated under diminished pressure at 45 °C for 3 hrs. The temperature was then raised to 60 °C to evaporate DMF until about 25 mL of reaction solution

remained. The solution was added to a mixture of aqueous saturated $NaHCO_3$-diethyl ether-ice in a separatory funnel, and extracted the aqueous phase with diethyl ether (4x). Standard processing of ether extracts provided a syrup which was dried under high vacuum overnight, then submitted to next step without further purification. δ_H (acetone-d_6) 1.24-1.35 (6H, m, CH_3); 3.81 (3H, s, OCH_3); 4.22-4.30 (4H, m, CH_2); 4.84 (1H, d, $J_{2,3}$ 3.8, H-2 or H-3); 4.98 (1H, d, $J_{2,3}$ 3.8, H-2 or H-3); 6.06 (1H, s, benzylidene H); 6.89-7.56 (4 H, m, aromatic H); δ_C (acetone-d_6) 14.34, 14.36 (CH_3), 55.56 (OCH_3), 62.18, 62.22 (OCH_2), 77.84 and 78.34 (C-2 and C-3), 107.17 (benzylidene C), 114.31, 129.77, 132.44 and 161.85 (aromatic C).

The syrup was dissolved in abs. EtOH (60 mL) and added to the stirred solution of sodium borohydride (1.70 g, 44.8 mmol) in abs. EtOH (45 mL) at 0 °C. The solution was stirred for 2.5 hours, then evaporated. The residue was dissolved in EtOAc, washed with brine (4x), and processed to afford a syrup which was purified by silica gel chromatography (hexane-EtOAc, 2:1; then EtOAc) to provide (2S,3S)-2,3-*O*-(4-*O*-methoxybenzylidene) threitol (5.63 g, 90% yield based on diethyl L-tartrate). Alternatively, the crude product obtained prior to silica gel chromatography could be submitted to the next step. δ_H (D_2O-acetone-d_6, 1:9) 3.65-3.73 (4H, m, H-1 and H-4); 3.75 (3H, s, OCH_3); 3.98-4.07 (2H, m, H-2 and H-3); 5.84 (1H, s, benzylidene H); 6.87-7.39 (4 H, m, aromatic H); δ_C (D_2O-acetone-d_6, 1:9) 55.45 (OCH_3), 62.61, 62.93 (C-1 and C-4), 79.97 and 80.40 (C-2 and C-3), 103.97 (benzylidene C), 114.06, 128.98, 131.02 and 161.03 (aromatic C).

A mixture of (2S,3S)-2,3-*O*-(4-*O*-methoxybenzylidene)threitol (5.38 g, 22.4 mmol), benzyl bromide (11.48 g, 67.13 mmol) and powdered KOH (8.79 g, 156.6 mmol) in toluene (47 mL) was refluxed 24 hrs. The reaction mixture was filtered through Celite, washed with toluene, and evaporated to give a syrup which was purified by silica gel

chromatography (hexane-EtOAc, 6:1) to provide **16** (7.38 g, 79%). δ_H (acetone-d_6) 3.71-3.73 (4H, m, H-1 and H-4); 3.78 (3H, s, OCH_3); 4.18-4.27 (2H, m, H-2 and H-3); 4.59 and 4.60 (4H, s, benzylic H); 5.90 (1H, s, benzylidene H); 6.89-7.43 (14 H, aromatic H); δ_C (acetone-d_6) 55.51 (OCH_3), 71.44, 71.45 (benzylic C), 73.73 and 73.75 (C-1 and C-4), 78.46 and 78.97 (C-2 and C-3), 104.54 (benzylidene C), 113.56, 114.20, 128.20, 128.26, 128.29, 129.04, 129.08, 129.13, 131.21, 139.49 and 163.55 (aromatic C).

*(2S,3S)-1,4-dibenzyloxy-3-(4-methoxybenzyloxy)-2-butanol (**17**).* Diisobutylaluminum hydride (41.8 mmol, 28 mL of 1.5M solution) was added dropwise to the stirred solution of **16** (7.03 g, 16.7 mmol) in toluene (50 mL) at 0 °C. The reaction mixture was stirred for 4 hrs at the same temperature, at which time saturated NH_4OH (30 mL) and THF (80 mL) were added. The mixture was stirred for 2 hrs, filtered through Celite, washed with THF, evaporated and dried under high vacuum to provide **17** as a syrup (6.86 g, 97%) which is pure enough for next step. δ_C (acetone-d_6) 55.43 (OCH_3), 71.11 (C-2), 71.14, 72.17, 73.10 (benzylic C), 73.58 and 73.68 (C-1 and C-4), 78.82 (C-3), 114.29, 128.11, 128.25, 128.39, 128.98, 129.01, 130.19, 131.99, 139.72, 139.74 and 160.07 (aromatic C).

*(2S,3S)-1,4-dibenzyloxy-3-(4-methoxybenzyloxy)-2-butyl mesylate (**18**).* Mesyl chloride (2.78 g, 24.4 mmol) was added to a stirred solution of compound **17** (6.86 g, 16.24 mmol) in pyridine at 0 °C. The mixture was stirred at ambient temperature for 2 days, then diluted with dichloromethane, washed with cold 3% HCl (5 x). Standard processing afforded a syrup which was purified by silica gel chromatography (hexane-EtOAc, 2:1) to provide **18** (7.97 g, 15.9 mmol, 98%). δ_H (acetone-d_6) 3.04 (3H, s, CH_3SO_2); 3.65 (1H, dd, $J_{4a,\,3}$ 5.2, $J_{4a,\,4b}$ 10.4, H-4a); 3.73 (1H, dd, $J_{4b,\,3}$ 4.6, $J_{4a,\,4b}$

10.4, H-4b); 3.73-3.79 (2H, m, H-1a and H-1b); 3.77 (3H, s, OCH_3); 3.91 (1H, m, H-3); 4.48-4.66 (6 H, m, benzylic H); 4.90-4.94 (1H, m, H-2), 6.88-7.48 (18 H, m, aromatic H); δ_C (acetone-d_6) 38.58 (CH_3SO_2), 56.46 (OCH_3), 69.38 (C-4), 69.91 (C-1), 73.15, 73.71, 73.80 (benzylic C), 77.39 (C-3), 81.85 (C-2), 114.41, 128.29, 128.45, 128.50, 128.66, 129.08, 129.14, 130.47, 131.22, 138.92, 139.32 and 160.29 (aromatic C).

*(2R,3S)-1,4-Dibenzyloxy-3-(4-methoxybenzyloxy)-2-(2-methoxyphenoxy)butane (**19**).* Cesium 2-methoxyphenolate (4.77 g, 18.6 mmol) and 18-crown-6 (3.90 g, 14.8 mmol) were added to a solution of **18** (4.10 g, 7.45 mmol) in dry benzene (40 mL). The mixture was stirred at reflux (80°C) for 24 hrs, filtered through Celite, and evaporated to provide a syrup. The syrup was purified by silica gel chromatography (hexane-ethyl acetate; 6:1) to provide **19** (3.91 g, 85-90% purity). The byproducts were difficult to remove by silica gel column chromatography. However, these byproducts were readily eliminated after the subsequent deprotection step. A small amount of pure **19** was obtained through the purification of the syrup by preparative TLC (hexane-ethyl acetate; 6:1). $[\alpha]_D$ -13.8 (c 1.44, in $CHCl_3$); δ_H (acetone-d_6) 3.72 (1H, dd, $J_{3,\,4a}$ 5.5, $J_{4a,\,4b}$ 10.4, H-4a); 3.76 (3H, s, OCH_3); 3.78 (3H, s, OCH_3); 3.80 (1H, dd, $J_{1a,\,2}$ 5.2, $J_{1a,\,1b}$ 10.5, H-1a); 3.84 (1H, dd, $J_{3,\,4b}$ 4.1, $J_{4a,\,4b}$ 10.4, H-4b); 3.87 (1H, dd, $J_{1b,\,2}$ 3.6, $J_{1a,\,1b}$ 10.5, H-1b); 4.03 (1 H, dt, $J_{3,\,4b}$ 4.1, $J_{3,\,4a}$, $J_{3,\,2}$ 5.5, H-3); 4.44-4.55 (4 H, m, benzylic H), 4.60-4.70 (3 H, m, H-2, benzylic H), 6.81-7.30 (18 H, m, aromatic H); δ_C (acetone-d_6) 55.42, 56.13 (OCH_3), 69.92 (C-1), 70.74 (C-4), 72.94, 73.65 (benzylic C), 78.30 (C-3), 79.78 (C-2), 113.56, 114.28, 118.14, 121.56, 122.73, 128.07, 128.24, 128.26, 128.98, 130.13, 131.86, 139.65, 139.69, 148.91, 151.64 and 160.05 (aromatic C).

*(2R,3S)-3-(2-methoxyphenoxy)-1,2,4-butanetriol (**20**).* Pd-C (10% by wt, 0.39 g) was added to a stirred solution of crude **19** (3.91g) in 36 mL of methanol-formic acid (9:1). A

balloon filled with hydrogen gas was placed on the top of the flask. The reaction mixture was stirred vigorously until TLC showed that reaction was complete. The reaction mixture was then filtered through a 0.2 μm nylon membrane filter, and the filtrate was evaporated to provide a syrup which was purified by silica gel chromatography (chloroform-methanol, 6:1) to provide **20** (1.29 g, 76% based on **18**). $[\alpha]_D$ -29.3 (c 1.05, in MeOH); δ_H (D_2O-acetone-d_6, 20:80) 3.60 (1H, dd, $J_{2,\ 1a}$ 6.3, $J_{1a,\ 1b}$ 11.6, H-1a); 3.71 (1H, dd, $J_{1b,\ 2}$ 4.2, $J_{1a,\ 1b}$ 11.6, H-1b); 3.74-3.82 (2H, m, H-4a, H-4b); 3.76 (3H, s, OCH_3); 3.95 (1 H, dt, $J_{2,\ 1b}$ 4.3, $J_{2,\ 1a}$, $J_{3,\ 2}$ 6.1, H-2); 4.22-4.27 (4 H, m, H-3, OH-1, 2, 4); 6.80-7.10 (4 H, m, aromatic H). δ_C (D_2O-acetone-d_6, 20:80) 55.97 (OCH_3), 60.42 (C-1), 63.40 (C-4), 74.44 (C-2), 81.26 (C-3), 112.99, 117.41, 121.71, 122.73, 147.71 and 150.54 (aromatic C).

(2R)-3-hydroxy-2-(methoxyphenyloxy)propyl aldehyde (21). Lead tetraacetate (1.20 g, 2.70 mmol) was added to a solution of **20** (0.59 g, 2.59 mmol) in dry benzene (15 mL) under an atmosphere of nitrogen. The reaction mixture was refluxed for 4 hrs, then filtered through a pipet clogged with glass wool. The filtrate was evaporated, dried under high vacuum for 1 hour to provide a syrup **21** which was immediately submitted to next step.

(2R,1RS)-2-(2-methoxyphenoxy)-1-phenyl-1,3-propanediol (22 and 23). A solution of **21** generated from the above step in THF (9 mL) was added to stirred phenyl lithium (5.8 mL of 1.8 M solution, 10.37 mmol) cooled in an ice-water bath under an atmosphere of nitrogen. The reaction mixture was stirred for 6 hrs at the same temperature, then poured into cold aqueous NH_4Cl and extracted with chloroform (4x). Standard processing provided a syrup which was purified by silica gel chromatography ($CHCl_3$-EtOAc, 1:1) to afford an equal amount of **22** and **23** (0.511 g, 72% based on **20**). Compounds, **22** and **23**, were separated with ion exchange chromatography (QAE Sephadex, A-25)[11] using

0.06 M $K_2B_4O_7$ in acetone-water (1:4) as the eluent to provide **22** (0.221 g) and **23** (0.244 g). (2R,1R)-2-(2-methoxyphenoxy)-1-phenyl-1,3-propanediol **22**: $[\alpha]_D$ -98.6 (c 1.18, in $CHCl_3$); δ_H (acetone-d_6) 3.47 (1H, m, H-3a); 3.69 (1H, ddd, $J_{3a,\ 3b}$ 11.9, $J_{OH-3,\ 3b}$ 3.7, $J_{2,\ 3b}$ 6.0, H-3b); 3.84 (1H, OH-3); 3.85 (3H, s, OCH_3); 4.20 (1H, dt, $J_{2,\ 3a}$ 3.8, $J_{2,\ 3b}$, $J_{2,\ 1}$ 6.1, H-2); 4.52 (1H, d, $J_{1\text{-}OH,\ 1}$ 3.8, OH-1); 4.99 (1 H, dd, $J_{2,\ 1}$ 6.3, $J_{1\text{-}OH,\ 1}$ 3.8, H-1); 6.62-7.50 (9 H, aromatic H); δ_C (acetone-d_6) 56.24 (OCH_3), 61.69 (C-3), 73.92 (C-1), 88.47(C-2), 113.39, 120.27, 121.89, 123.60, 127.87, 128.13, 128.70, 142.52, 149.54 and 151.87 (aromatic C). (2R,1S)-2-(2-methoxyphenoxy)-1-phenyl-1,3-propanediol **23**: $[\alpha]_D$ -5.3 (c 0.97, in $CHCl_3$); δ_H (acetone-d_6) 3.70 (1H, dd, $J_{3a,\ 2}$ 3.6, $J_{3a,\ 3b}$ 12.0, H-3a); 3.75 (3H, s, OCH_3); 3.86 (1H, dd, $J_{3a,\ 3b}$ 12.0, $J_{2,\ 3b}$ 5.3, H-3b); 4.33 (1H, m, H-2); 4.97 (1 H, dd, $J_{2,\ 1}$ 5.6, H-1); 6.76-7.45 (9 H, m, aromatic H); δ_C (acetone-d_6) 56.10 (OCH_3), 60.92 (C-3), 73.05 (C-1), 85.45(C-2), 113.24, 118.31, 121.70, 122.95, 127.48, 127.78, 128.48, 142.40, 148.22 and 151.09 (aromatic C).

7.6 References

1. R. S. Ward, *Nat. Prod. Rep.*, **1995**, 183.
2. K. B. Sharpless, W. Amberg, Y. L. Bennani, G. A. Crispino, J. Hartung, K. S. Jeong, H. L. Kwong, K. Morikawa, Z. M. Wang, D. Xu and X. L. Zhang, *J. Org. Chem.*, 57 (**1992**) 2769.
3. J. Denis, A. Correa and A. E. Greene, *J. Org. Chem.*, 55 (**1990**) 1959.
4. S. A. Zacchino, *J. Nat. Prod.*, 57 (**1994**) 446.
5. S. K. Kang, D. C. Park, H. S. Rho, S. H. Yoon and J. S. Shin, *J. Chem. Soc. Perkin Trans 1.*, **1994**, 3513.
6. a) R. M. Burk and M. B. Roof, *Tetrahedron Lett.*, 34 (**1993**) 395.
   b) S. D. Kang, J. H. Jeon, K. S. Nam, C. H. Park and H. W. Lee, *Synth.*

Commun., 24 (**1994**) 305.

7. O. Mitsunobu, *Synthesis*, **1981**, 1.
8. R. Johansson and B. Samuelsson, *J. Chem. Soc. Perkin Trans. 1*, **1984**, 2371.
9. R. M. Wenger, *Helv. Chim. Acta*, 66 (**1983**) 2308.
10. S. Takano, A. Kurotaki, Y. Sekiguchi, S. Satoh, M. Hirama and K. Ogasawara, *Synthesis*, **1986**, 811.
11. W. Ibrahim and K. Lundquist, *Acta Chem. Scand.*, 48 (**1994**) 149.

Chapter 8

The preparation of Chiral Aryl Alkyl Ethers

8.1 Abstract

A new approach to chiral aryl alkyl ethers has been developed by using cesium phenolate and 18-crown-6. The use of this new method on a tartrate derivative has resulted in the preparation of several 8-*O*-4' neolignans, as well as a benzodioxane neolignan. These compounds have greater than 96% enantiomeric excess, and this work represents the first successful preparation of optically active 8-O-4' type neolignans. The key chiral aryl alkyl ether bond was introduced into several carbohydrate molecules, which serve as the chiral auxiliary for the propane side chain.

8.2 Introduction

Neolignans are an important class of chiral secondary metabolites in plant tissues having a very broad structural variation.[1] In the classical definition, lignans are optically active dimers composed of lignin monomers linked 8-8', that may or may not have undergone post-coupling modifications. Neolignans, on the other hand, are optically active arylpropane dimers linked by any other viable free radical bond coupling process, besides 8-8'. Neolignans, especially 8-*O*-4'

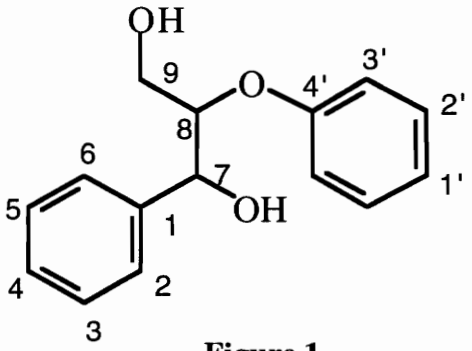


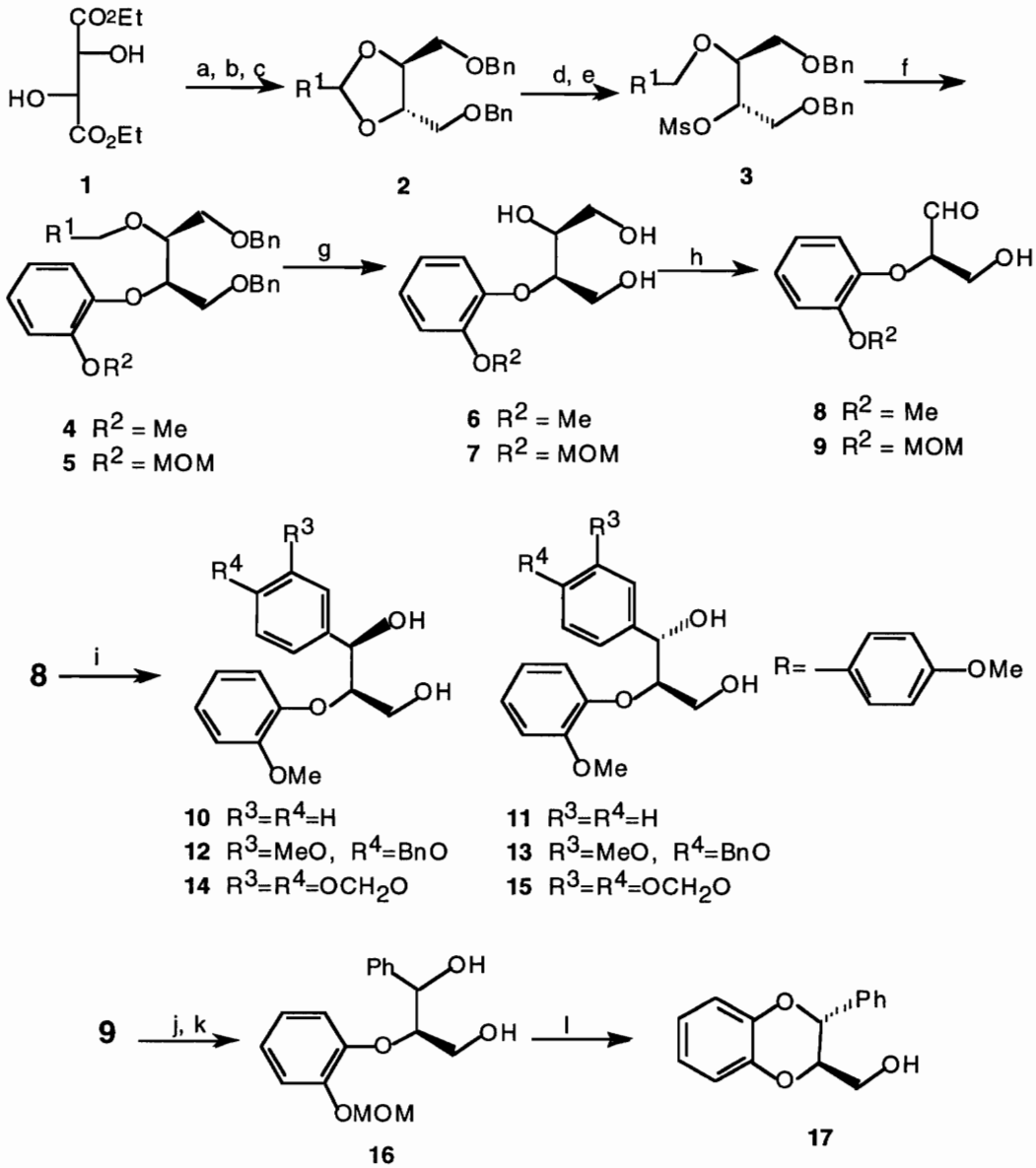
Figure 1

neolignans (Figure 1), are structurally analogous to the interunit linkages of lignin. Lignin is known as an optically inactive polymer, whereas neolignans are optically active small molecules. The biosynthetic relationship between polymeric lignin and small chiral neolignans is still unclear. In addition, 1,4-benzodioxane structures have been found in a broad array of lignans and neolignans,[1] some of which are biologically active and have potent insecticidal activities.[2-6] It is also possible to find sweet compounds in the 1,4-benzodioxane class.[7] In an effort to define the biosynthetic pathways and the functions of neolignans in the plant cell wall, we synthesized optically active 8-*O*-4 and benzodioxane neolignans.

The 8-*O*-4' and benzodioxane neolignans contain a chiral aryl alkyl ether bond. The construction of this chiral ether bond is the key step in the enantiomeric synthesis of these compounds, and synthetic methods for the preparation of a chiral aryl alkyl ether bond have been scarce in the literature. Described herein is a novel synthetic method to prepare chiral aryl alkyl ether bonds which was optimized during our synthesis of several 8-*O*-4' and benzodioxane neolignans. In order to test the versatility of this new method, several carbohydrate derivatives were also used as substrates. Because chiral aryl alkyl ether bonds exist in a broad array of natural products,[1,5,6] this new method may find widespread application. Moreover, a method to construct a chiral aryl alkyl ether bond in carbohydrate molecules allows for use of inexpensive and readily available chiral templates.

8.3 Results and Discussion

As described in Chapter 7, several synthetic routes to 8-*O*-4' neolignans have been attempted. These studies revealed that the best overall strategy is to initially prepare a chiral aryloxy ether bond by using the natural chiral centers in diethyl tartrates, then to



Scheme 1. *Reagents and conditions:* a, 4-methoxybenzaldehyde dimethyl acetal/*p*-TsOH/DMF; b, $NaBH_4$/EtOH; c, BnBr/KOH/toluene; d, DIBAL-H/toluene; e, MsCl/pyridine; f, cesium phenolate/18-crown-6; g, H_2/Pd-C/MeOH:HCOOH; h, $Pb(OAc)_4$/benzene; i, ArLi/THF; j, MeOH/H^+; k, PhLi/THF; l, Amberlyst 15

introduce an aromatic ring at the 7-position (Scheme 1). Protection, reduction and benzylation of diethyl L-tartrate **1** provided **2** in 71% yield.[9-11] Reductive ring-opening of

2 and subsequent mesylation afforded **3** in 95% yield.[11] The substitution reaction of **3** with sodium 2-methoxyphenolate in refluxing benzene for 30 hrs only provided a trace amount of **4**. The addition of 18-crown-6 accelerated the substitution reaction; and TLC showed that about 30% of **3** was converted to **4** when 18-crown-6 was added to the reaction mixture, and refluxed for 30 hrs. Cesium phenolate was superior to sodium phenolate. Compound **3** completely disappeared when the mixture of **3**, cesium 2-methoxy phenolate and 18-crown-6 was refluxed in dry benzene for 24 hrs. The reaction of **3** with cesium 2-(methoxymethoxy)phenolate in the presence of 18-crown-6 also provided **5**. Compound **6** was prepared by hydrogenation of **4** with Pd-C in methanol-formic acid (9:1) in 76% yield based on **3**. Similarly, hydrogenation of **5** with Pd-C in methanol and a catalytic amount of formic acid provided **7** in 60% yield based on **3**. Criege glycol oxidation of **6** and **7** with lead tetraacetate in dry benzene afforded **8** and **9**, respectively. Compounds **8** and **9** were submitted to next step reaction without further purification.

It is noteworthy that compounds **8** and **9** easily polymerized, especially in the presence of water. A ^{13}C NMR spectrum of crude product generated from diol cleavage of **6** with sodium periodate in an aqueous medium showed more than 30 peaks in 60-100ppm, and only a weak carbonyl signal of aldehyde.[12]

The reaction of **8** with phenyl lithium afforded equal amounts of two diastereomers, **10** and **11**, in 72% yield based on **6**. The coupling of phenyl lithium with the product generated from sodium periodate oxidation of **6** did not afford **10** and **11**. Two diastereomers, **12** and **13**, **14** and **15**, were prepared by the coupling of **8** with the corresponding aryl lithium. The resulting two diasteromers were separated by ion exchange chromatography (QAE-Sephadex A-25) using 0.06 M $K_2B_4O_7$ in acetone-water (1:4) as the eluent.[13]

Enantiomeric excess of the products obtained was accomplished by preparation of Mosher esters. Compounds **10-15** have greater than 96% ee as determined by NMR of the bis-Mosher esters of **10-15**, which were prepared in almost quantitative yield by using the Keck modification[14] of Steglich esterification. Bis-Mosher esters of **12** and **13** were separated by preparative TLC. Small amounts of **10-15** could also be prepared by conversion of **10-15** to bis-Mosher esters, separation of the two esters, and subsequent saponification with MeONa/MeOH.

The reaction of **9** with phenyl lithium provided **16** (two diastereomers) in 70% yield based on **9**. The deprotection of **16** in methanol with a catalytic amount of HCl, and subsequent cyclization with Amberlyst 15 in dry toluene provided **17** in 53% yield. Compound **17** has greater than 96% ee as determined by NMR of the Mosher ester of **17**.

Compounds, **10-15**, have an 8R-configuration in the phenylpropane sidechain. The use of diethyl D-tartrate as a starting material allows the preparation of 8-*O*-4' neolignans having an 8S-configuration and the enantiomer of **17**.

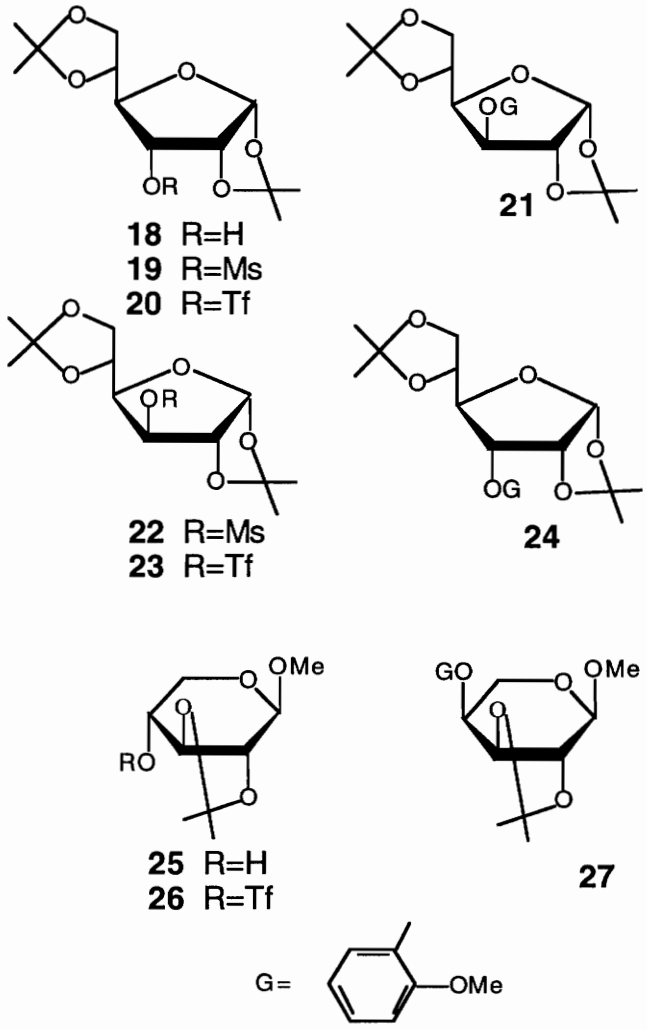


Figure 2

The synthetic method to prepare chiral aryl alkyl ethers was applied to several carbohydrates (Figure 2). Only small amount of **21** (about 3% yield) was isolated when the mixture of **19**, cesium 2-methoxyphenolate and 18-crown-6 in benzene was refluxed for 2 hrs. The starting material was recovered in 85% yield. However, the reaction of **20** with cesium 2-methoxyphenolate and 18-crown-6 in benzene afforded **21** in 95% yield based on **18**. Selective diol cleavage between C-1 and C-2 of **21**, and subsequent aryl lithium coupling allow entry into two diastereomers of 8-*O*-4' neolignans having an 8S-configuration, whereas selective diol cleavage between C-4 and C-5 allows the preparation of the two diastereomers having an 8R-configuration. Compound **24** was obtained in low yield when **22** or **23** was reacted with cesium 2-methoxyphenolate and 18-crown-6 in benzene. This is because 1, 2-isopropylidene ring hindered the phenolate anion attack. The reaction of **26** with cesium 2-methoxyphenolate, 18-crown-6 in DMF quickly provided **27** in 64% yield based on **25**. Successful construction of an aryl alkyl ether bond in carbohydrate molecules demonstrates that this method may be used in the preparation of other natural products where chiral alkyl aryl ether bonds are required. Carbohydrates are readily available, and have well-defined chiral centers. A method to introduce an alkyl aryl ether bond in carbohydrate molecules allows us to make good use of the natural chiral centers in carbohydrates, and allows the use of carbohydrates as starting materials for the preparation of natural products. For example, chiral propane sidechains of 8-O-4 and benzodioxane neolignans can be prepared from carbohydrates by the introduction of an chiral aryl alkyl ether bond in a carbohydrate molecule, and subsequent controlled diol cleavage with lead tetraacetate. Moreover, This new method also allows easy entry into chiral substrates for Claisen rearrangements, *e.g.* the conversion of 2,3-diol in **27** into a double bond allows the preparation of an substrate for Claisen rearrangement.

8.4 Conclusions

A new method of preparing chiral aryl alkyl ether bonds has been developed by using cesium phenolate and 18-crown-6. Two cesium phenolates were successfully used in the preparation of chiral aryl alkyl ethers from a tartrate derivative. Chiral 8-*O*-4' neolignans, **10-15**, and a chiral benzodioxane neolignan, **17**, were prepared by the use of this new method. The prepared neolignans have greater than 96% enantiomeric excess. This new method was successfully used to introduce a chiral alkyl aryl ether bond in carbohydrate molecules. The successful introduction of a chiral alkyl aryl ether bond in a tartrate derivative and carbohydrates demonstrated that this method may have broad applications. This new method is not only useful in the synthesis of natural products having a chiral alkyl aryl ether bond, but also allows easy entry into the chiral substrates of Claisen rearrangement.

8.5 Experimental

Air and moisture sensitive reactions were performed under an atmosphere of dry nitrogen. Methylene chloride, benzene and toluene were distilled from CaH_2. THF was distilled from Na. Pyridine was distilled from KOH. Other reagents were obtained from commercial sources and used without further purification. Evaporation were performed under diminished pressure at temperature not exceeding 42 °C. Standard process implies that organic solvents were dried with Na_2SO_4 or $MgSO_4$, filtered and evaporated. Drying of products was accomplished under high vacuum (25-75 mTorr). Silica gel chromatography was performed using silica gel 60 (230-400 mesh, Whatman). Thin layer chromatography (TLC) was performed with Alugram Sil-G/Uv254 plates (Macherey-Nagel) with UV light or by charring (5% H_2SO_4 in 95% EtOH). Preparative TLC was performed on Silica-G/glass (Whatman). NMR spectra were recorded at 400 MHz (Varian

Unity 400) at ambient temperature with acetone-d_6 as solvent (unless indicated otherwise) and internal reference (central solvent peak, δ_H, 2.04 ppm; δ_C, 29.8 ppm) .

*(2S,3S)-1,4-Di-O-benzyl-2,3-(4-methoxybenzylidene)threitol (**2**).*[9-11] As described in the preparation of **16** in Chapter 7.

*(2S, 3S)-1,4-dibenzyloxy-3-(4-methoxybenzyloxy)-2-butyl mesylate (**3**).* As described in the preparation of **18** in Chapter 7.

*(2R,3S)-1,4-Dibenzyloxy-3-(4-methoxybenzyloxy)-2-(2-methoxyphenoxy)butane (**4**).* As described in the preparation of **19** in Chapter 7.

*(2R,3S)-3-(2-methoxyphenoxy)-1,2,4-butanetriol (**6**).* As described in the preparation of **20** in Chapter 7.

*(2R,3S)-1,4-Dibenzyloxy-3-(4-methoxybenzyloxy)-2-(2-(methoxymethoxy)phenoxy)butane (**5**).* According to the procedure used in the preparation of **4**, compound **5** was prepared from the reaction of **3**, cesium 2-methoxymethoxyphenolate and 18-crown-6. δ_H (acetone-d_6) 3.41 (3H, s, OCH_3); 3.74 (1H, dd, $J_{3,\ 4a}$ 5.5, $J_{4a,\ 4b}$ 10.4, H-4a); 3.76 (3H, s, OCH_3); 3.82 (1H, dd, $J_{1a,\ 2}$ 5.3, $J_{1a,\ 1b}$ 10.6, H-1a); 3.85 (1H, dd, $J_{3,\ 4b}$ 4.1, $J_{4a,\ 4b}$ 10.4, H-4b); 3.88 (1H, dd, $J_{1b,\ 2}$ 3.6, $J_{1a,\ 1b}$ 10.6, H-1b); 4.05 (1 H, dt, $J_{3,\ 4b}$ 4.1, $J_{3,\ 4a}$, $J_{3,\ 2}$ 5.3, H-3); 4.47-4.71 (7 H, m, H-2, benzylic H); 5.12-5.16 (2 H, m, OCH_2O), 6.74-7.36 (18 H, m, aromatic H); δ_C (acetone-d_6) 55.42, 56.22 (OCH_3), 69.82 (C-1), 70.59 (C-4), 72.93, 73.66 (benzylic C), 78.27 (C-3), 79.58 (C-2), 96.38 (OCH_2O), 114.29, 118.01, 118.98, 122.45, 123.42, 128.10, 128.24, 128.26, 128.98, 130.10, 131.77, 139.55, 139.61, 148.68, 149.95 and 160.05 (aromatic C).

(2R,3S)-3-(2-(methoxymethoxy)phenoxy)-1,2,4-butanetriol (7). As described in the preparation of **6**, hydrogenation of crude **5** generated from **3** (2.08 g, 3.77 mmol) in methanol (22 mL) and formic acid (0.2 mL) with Pd-C (10% by wt, 0.2 g) provided **7** (0.58 g, 60% based on **3**). $[\alpha]_D$ -29.4 (c 1.14, in MeOH). δ_H (D_2O-acetone-d_6, 20:80) 3.43 (3H, s, OCH_3); 3.63 (1H, dd, $J_{2,\ 1a}$ 6.1, $J_{1a,\ 1b}$ 11.5, H-1a); 3.75 (1H, dd, $J_{1b,\ 2}$ 4.3, $J_{1a,\ 1b}$ 11.5, H-1b); 3.80 (1H, dd, $J_{3,\ 4a}$ 4.6, $J_{4a,\ 4b}$ 12.1, H-4a); 3.84 (1H, m, H-4b); 3.96 (1 H, dt, $J_{2,\ 1b}$ 4.3, $J_{2,\ 1a}$, $J_{3,\ 2}$ 6.1, H-2); 4.30 (1 H, dt, $J_{3,\ 4a}$ 4.4, $J_{3,\ 4b}$, $J_{3,\ 2}$ 5.8, H-3); 5.16 (2H, m, OCH_2O); 6.84-7.15 (4 H, m, aromatic H). δ_C (D_2O-acetone-d_6, 20:80) 56.31 (OCH_3), 60.76 (C-4), 63.59 (C-1), 71.77 (C-3), 81.56 (C-2), 96.18 (OCH_2O), 117.98, 118.43, 122.48, 123.56, 148.12 and 149.36 (aromatic C).

(2R)-3-hydroxy-2-(methoxyphenyloxy)propyl aldehyde (8). As described in the preparation of **21** in Chapter 7.

(2R,1RS)-2-(2-methoxyphenoxy)-1-phenyl-1,3-propanediol (10 and 11). As described in the preparation of **22** and **23** in Chapter 7.

1-benzyloxy-4-bromo-2-methoxybenzene. 4-bromo-2-methoxyphenol was prepared according to the literature procedure[15]. A mixture of 4-bromo-2-methoxyphenol (0.352 g, 1.736 mmol), benzyl bromide (0.312 g, 1.82 mmol) and powdered K_2CO_3 (0.26 g, 1.91 mmol) in acetone (5 mL) was stirred and refluxed overnight. The mixture was then filtered, evaporated and purified with silica gel chromatography (hexane-EtOAc, 39:1) to provide a white solid which crystallized from 95% EtOH to provide the compound as long needles (0.412 g, 81%). mp. 59-60 °C. δ_H (acetone-d_6) 3.84 (3H, s, OCH_3); 5.10 (2H, s, benzylic H); 6.94-7.48 (8 H, aromatic H).

*(2R,1RS)-1-(4-benzyloxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1, 3-propanediol (**12** and **13**).* A solution of 1-benzyloxy-4-bromo-2-methoxybenzene (0.48 g, 1.64 mmol) in THF (5 mL) was stirred and cooled to -60 °C, and n-BuLi (1.64 mmol, 0.66 mL of 2.5 M solution) was added under an atmosphere of nitrogen. To the reaction mixture was added a solution of **8** generated from **6** (0.0938 g, 0.411 mmol) in THF (4 mL). The reaction mixture was warmed to 0 °C and stirred for 6 hrs, poured into cold aqueous NH_4Cl, and extracted with chloroform (4x). Standard processing provided a syrup which was purified by silica gel chromatography ($CHCl_3$-EtOAc, 1:1) to give a mixture of **12** and **13** (0.0595 g, 35.3%).

A mixture of **12** and **13** (0.0246 g, 0.0599 mmol), (R)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid [(+)MTPA] (0.042 g, 0.18 mmol), 1,3-dicyclohexylcarbodiimide (DCC) (0.037 g, 0.18 mmol), 4-dimethylaminopyridine (DMAP) (0.022 g, 0.18 mmol), DMAP•HCl (0.029 g, 0.18 mmol) in CH_2Cl_2 (2 mL) was stirred at room temperature for 24 hours.[14] The reaction mixture was filtered through a glass wool plug, and the filtrate was diluted with $CHCl_3$, and washed with cold 3% HCl (1x). Standard processing provided a syrup which was purified by preparative TLC (petroleum ether-diethyl ether, 80:20, developed four times) to afford the bis-Mosher ester of **12** (0.0241 g) and **13** (0.0235 g) in 94.2% yield. A solution of the bis-Mosher ester of **12** (0.0241 g, 0.0285 mmol) and sodium methoxide (3 mg) in methanol (3 mL) was stirred at room temperature overnight. The solution was neutralized with ion exchange resin, filtered, and evaporated to provide a syrup which was purified by a preparative TLC ($CHCl_3$-ETOAc, 1:1) to afford **12** (0.0114 g, 98%). Compound **13** (0.0112 g) was prepared in the same fashion from the bis-Mosher ester of **13** (0.0235 g). This procedure is useful for preparing small amounts of **12** and **13** due to difficulty associated with detecting small amounts of **12** and **13** in the separation ion exchange chromatography. Alternatively, a mixture of **12** and **13** (0.100 g) was submitted to ion exchange

chromatography using 0.06 M $K_2B_4O_7$ in acetone-water (1:4) as the eluent to provide **12** (0.0333 g) and **13** (0.0328 g). (2R,1R)-1-(4-benzyloxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol **12**: $[\alpha]_D$ -66.8 (c 1.47, in $CHCl_3$). δ_H (acetone-d_6) 3.49 (1H, m, H-3a); 3.69 (1H, ddd, $J_{3b,\ 2}$ 6.0, $J_{3b,\ OH\text{-}3}$ 3.7, $J_{3a,\ 3b}$ 11.9, H-3b); 3.79 (3H, s, OCH_3); 3.85 (3H, s, OCH_3); 3.86 (1H, m, OH-3); 4.20 (1H, dt, $J_{2,\ 3a}$ 3.8, $J_{2,\ 3b}$, $J_{2,\ 1}$ 6.0, H-2); 4.49 (1H, d, $J_{1\text{-}OH,\ 1}$ 3.8, OH-1); 4.91 (1 H, dd, $J_{2,\ 1}$ 6.3, $J_{1\text{-}OH,\ 1}$ 3.8, H-1); 5.08 (2H, s, benzylic H); 6.83-7.49 (7 H, m, aromatic H); δ_C (acetone-d_6) 56.00 (OCH_3), 56.18 (OCH_3), 61.77 (C-3), 71.29 (benzylic C), 73.64 (C-1), 88.26 (C-2), 111.94, 113.23, 114.38, 119.82, 119.95, 121.86, 123.36, 128.40, 128.45, 129.11, 135.58, 138.60, 148.58, 149.54, 150.44 and 151.69 (aromatic C). (2R,1S)-1-(4-benzyloxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol **13**: $[\alpha]_D$ -11.7 (c 1.55, in $CHCl_3$). δ_H (acetone-d_6) 3.69 (2H, m, H-3a, H-3b); 3.80 (3H, s, OCH_3); 3.82(1H, m, OH-3); 3.86 (3H, s, OCH_3); 4.30 (1H, dt, $J_{2,\ 3a}$ 4.0,$J_{2,\ 3b}$, $J_{2,\ 1}$ 5.5, H-2); 4.61 (1H, d, $J_{1,\ 1\text{-}OH}$ 4.1, OH-1); 4.92 (1 H, t, $J_{2,\ 1}$,$J_{1,\ 1\text{-}OH}$ 5.1, H-1); 5.07 (2H, s, benzylic H); 6.79-7.50 (7 H, m, aromatic H); δ_C (acetone-d_6) 56.10 (OCH_3), 56.21 (OCH_3), 61.74 (C-3), 71.39 (benzylic C), 73.66 (C-1), 86.55 (C-2), 101.74 (OCH_2O), 112.12, 113.43, 114.51, 119.62, 119.98, 120.02, 121.82, 123.32, 128.38, 128.40, 128.45, 129.12, 136.09, 138.64, 148.49, 148.94, 150.46 and 151.90 (aromatic C).

*(2R,1RS)-2-(2-methoxyphenoxy)-1-(3,4-methylenedioxyphenyl)-1,3-propanediol (**14** and **15**).* A solution of 4-bromo-1, 2-(methylenedioxy)benzene (0. 351 g, 1.75 mmol) in THF (1.5 mL) was stirred and cooled to -78 °C, and n-BuLi (1.75 mmol, 0.7 ml of 2.5 M solution) was added dropwise. The solution was allowed to warm up to -40 °C during 30 minutes, then a solution of **8** prepared from from **6** (0.0797 g, 0.0349 mmol) was added. The reaction was stirred in an ice-water bath for 6 hrs. Work-up as described in the preparation of **12** and **13** provided a syrup which was purified by a preparative TLC to afford a mixture of **14** and **15** (0.0690 g, 62.1%). Separation of **14** and **15** was achieved

as described in the preparation of **12** and **13**. (2R,1R)-2-(2-methoxyphenoxy)-1-(3,4-methylenedioxyphenyl)-1,3-propanediol **14**: $[\alpha]_D$ -80.1 (c 0.76, in $CHCl_3$). δ_H (acetone-d_6) 3.47 (1H, dd, $J_{3a,\,2}$ 5.5, $J_{3a,\,3b}$ 11.9, H-3a); 3.68 (1H, m, H-3b); 3.81 (1H, m, OH-3); 3.86 (3H, s, OCH_3); 4.15 (1H, ddd, $J_{2,\,3a}$ 5.5, $J_{2,\,3b}$ 3.7, $J_{2,\,1}$ 6.3, H-2); 4.48 (1H, d, $J_{1\text{-OH},\,1}$ 3.8, OH-1); 4.90 (1 H, dd, $J_{2,\,1}$ 6.3, $J_{1\text{-OH},\,1}$ 3.7, H-1); 5.95 (2H, m, OCH_2O); 6.77-7.18 (7 H, m, aromatic H); δ_C (acetone-d_6) 56.26 (OCH_3), 61.72 (C-3), 73.77 (C-1), 88.41(C-2), 101.78 (OCH_2O), 108.28, 108.36, 113.42, 120.18, 121.16, 121.91, 123.55, 136.50, 147.74, 148.34, 149.56, 151.87 and 156.57 (aromatic C). (2R,1S)-2-(2-methoxyphenoxy)-1-(3,4-methylenedioxyphenyl)-1,3-propanediol **15**: $[\alpha]_D$ -13.7 (c 0.59, in $CHCl_3$). δ_H (acetone-d_6) 3.69 (2H, m, H-3a, H-3b); 3.79 (1H, OH-3); 3.81 (3H, s, OCH_3); 4.25 (1H, dt, $J_{2,\,3a}$ 3.9, $J_{2,\,3b}$, $J_{2,\,1}$ 5.4, H-2); 4.62 (1H, d, $J_{1,\,1\text{-OH}}$ 4.3, OH-1); 4.90 (1 H, dd, $J_{2,\,1}$ 5.2, $J_{1,\,1\text{-OH}}$ 4.3, H-1); 5.94 (2H, s, OCH_2O); 6.75-7.03 (7 H, m, aromatic H); δ_C (acetone-d_6) 56.25 (OCH_3), 61.69 (C-3), 73.67 (C-1), 86.72 (C-2), 101.74 (OCH_2O), 108.24, 108.31, 113.54, 119.93, 121.10, 121.85, 123.46, 137.03, 147.59, 148.30, 148.99 and 152.05 (aromatic C).

*(2R)-3-hydroxy-2-(2-methoxymethoxyphenoxy)propyl aldehyde (**9**).* As described in the preparation of **8**, compound **9** (0.140 g) was prepared from **7** (0.165 g, 0.638 mmol). Crude **9** was submitted to next step without purification and characterization.

*(2R,1RS)-2-(2-methoxymethoxyphenoxy)-1-phenyl-1,3-propanediol (**16**).* As described in the preparation of **10** and **11**, compound **16** (0.137 g, 70.4%) was prepared from crude **9** (0.140 g).

*(1R,2R)-2-(hydroxymethyl)-3-phenyl-1,4-benzodioxane (**17**).* Two drops of conc. HCl were added to a solution of compound **16** (0.020 g, 0.066 mmol) in methanol (2 mL).

The solution was stirred at 65 °C for one hour, evaporated, and then dried under high vacuum for 15 minutes. The resulting syrup was dissolved in toluene (2 mL), and Amberlyst 15 (30 mg) was added. The reaction mixture was stirred and refluxed for one hour, then filtered and evaporated to provide a syrup. Purification by preparative TLC (hexane-EtOAc, 2:1) gave **17** (0.0084 g, 52.8%). $[\alpha]_D$ -19.8 (c 0.43, in $CHCl_3$). δ_H (acetone-d_6) 3.46 (1H, dd, $J_{11a,\,2}$ 4.0, $J_{11a,\,11b}$ 12.1, H-11a); 3.74 (1H, dd, $J_{11b,\,2}$ 2.6, $J_{11a,\,11b}$ 12.1, H-11b); 4.09 (1H, ddd, $J_{2,\,11a}$ 4.0, $J_{2,\,11b}$ 2.6, $J_{2,\,3}$ 7.8, H-2); 5.08 (1H, d, $J_{2,\,3}$ 7.8, H-3); 6.83-7.53 (9 H, m, aromatic H); δ_C (acetone-d_6) 61.65 (C-11), 77.01 (C-3), 79.42 (C-2), 117.69, 117.73, 121.91, 122.16, 128.51, 129.34, 129.52 and 129.64 (aromatic C).

1,2; 5,6-diisopropylidene-3-(2-methoxyphenyl)-α-D-glucofuranoside (**21**). A solution of **18** (1.07 g, 4.12 mmol) in pyridine was stirred and cooled in an ice-water bath, and trifluoroacetic anhydride (1.51 g, 5.36 mmol) was added under an atmosphere of nitrogen. After 2.5 hours, the reaction solution was diluted with dichloromethane, poured into a mixture of ice and aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (4x). Standard processing provided a syrup **20** which was submitted to next step reaction after drying under high vacuum for 2 hrs. The triflate, cesium 2-methoxyphenolate (2.85 g, 11.12 mmol) and 18-crown-6 (2.18 g, 8.24 mmol) in dry benzene (35 mL) were stirred at room temperature for 18 hours. The reaction mixture was filtered through Celite and evaporated to provide a syrup which was purified by silica gel chromatography (150 mL of hexane, then hexane-EtOAc, 6:1) to afford **21** (1.43 g, 95% yield based on **18**). $[\alpha]_D$ -26.2 (c 1.53, in $CHCl_3$). δ_H (acetone-d_6) 1.27 (3H, s, CCH_3); 1.28 (3H, s, CCH_3); 1.35 (3H, s, CCH_3); 1.46 (3H, s, CCH_3); 3.82 (3H, s, $ArOCH_3$); 4.01 (1H, dd, $J_{6a,\,5}$ 6.1, $J_{6a,\,6b}$ 8.5, H-6a); 4.14 (1H, dd, $J_{6b,\,5}$ 6.3, $J_{6a,\,6b}$ 8.5, H-6b); 4.35 (1H, dd, $J_{3,\,4}$ 3.2, $J_{5,\,4}$ 6.1, H-4); 4.51 (1H, q, $J_{6a,\,5}$, $J_{6b,\,5}$ $J_{5,\,4}$ 6.3, H-5); 4.64-4.66 (2H, m, H-2, H-3); 5.96

(1H, d, $J_{1,2}$ 3.8, H-1); 6.89-7.06 (4 H, m, aromatic H); δ_C (acetone-d_6) 25.63, 26.39, 26.96, 27.02 ($C(CH_3)_2$), 56.21 (OCH_3), 67.08 (C-6), 73.67 (C-5), 81.25 (C-4), 82.64 (C-3), 83.06 (C-2), 106.17 (C-1), 109.09 and 112.23 (CMe_2), 113.65, 116.89, 121.64, 123.43, 147.61 and 151.42 (aromatic C).

*Methyl 2,3-isopropylidene-4-(2-methoxyphenyl)-α-L-arabinopyranoside (**27**).* Trifluoroacetic anhydride (0.927 g, 3.286 mmol) was slowly added to a stirred solution of **25** (0.516 g, 2.528 mmol) in pyridine (10 mL) at 0 °C under a steam of nitrogen. The reaction was complete in an hour. Work-up as described in the preparation of **21** provided a syrup which was submitted to next step after drying under high vacuum for 2 hrs. To the solution of the syrup in DMF (10 mL) was added cesium 2-methoxyphenolate (2.560 g, 0.0100 mmol) and 18-crown-6 (1.640 g, 6.200 mmol). After stirred at ambient temperature for 2 hours, the mixture was added to a mixture of Et_2O-aqueous $NaHCO_3$-ice in a separatory funnel and extracted the aqueous phase with Et_2O (4x). Standard processing of the combined ether extracts provided a syrup which was purified by silica gel chromatography (CH_2Cl_2, then $CHCl_3$-EtOAc, 19:1) to provide **27** (0.494 g, 62.0% yield based on **25**). $[\alpha]_D$ +15.3 (c 1.09, in $CHCl_3$). δ_H (acetone-d_6) 1.34, 1.36 (2x3H, s's, CCH_3); 3.46 (3H, s, 1-OCH_3); 3.68 (1H, dd, $J_{5a,4}$ 1.5, $J_{5a,5b}$ 13.0, H-5a); 3.75 (1H, dd, $J_{3,2}$ 9.7, $J_{3,4}$ 2.5, H-3); 3.81 (3H, s, $ArOCH_3$); 3.97 (1H, dd, $J_{2,3}$ 9.7, $J_{2,1}$ 7.7, H-2); 4.14 (1H, dd, $J_{5b,4}$ 1.8, $J_{5a,5b}$ 13.0, H-5b); 4.55 (1H, d, $J_{1,2}$ 7.8, H-1); 4.76 (1H, m, H-4); 6.82-7.10 (4 H, m, aromatic H); δ_C (acetone-d_6) 25.84 and 26.10 ($C(CH_3)_2$), 55.13 (OCH_3), 55.43 (OCH_3), 65.95 (C-5), 73.48 (C-2), 74.92 (C-3), 77.81 (C-4), 103.99 (C-1), 109.63 ($C(CH_3)_2$), 112.98, 119.09, 120.69, 122.68, 147.96 and 151.09 (aromatic C).

8.5 References

1. Ward, R. S. *Nat. Prod. Rep.* **1995**, 183.

2. Mittel, I; Pelter, A; Hänsel, R. *Chem. Ber.* **1975**, 108, 790.

3. Woo, W. S.; Kang, S. S.; Wagner, H.; Chari, V. M. *Tetrahedron Lett.* **1978**, 19, 3239.

4. Ray, A. B.; Charttopadhyay, S. K.; Kondo, C.; Hikino, H. *Tetrahedron Lett.* **1980**, 21, 4477.

5. Ishibashi, T.; Taniquchi, E. *Agric. Biol. Chem.* **1989**, 53, 1557.

6. Taniquchi, E.; Imamura, K.; Ishibashi, F.; Matsui, T.; Nishio, A. *Agric. Biol. Chem.* **1989**, 53, 631.

7. Arnoldi, A; Camarda, L.; Merlini, L. *J. Agric. Food Chem.* **1986**, 34, 339.

8 Li, K.; Helm, R. F. *J. Chem. Soc., Perkin Trans. 1* submitted.

9. Johansson, R.; Samuelsson, B. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2371.

11. Wenger, R. M. *Helv. Chim. Acta* **1983**, 66, 2308.

12. Takano, S.; Kurotaki, A.; Sekiguchi, Y.; Satoh, S.; Hirama, M.; Ogasawara, K. *Synthesis* **1986**, 811.

13. Ibrahim, W.; Lundquist, K. *Acta Chem. Scand.* **1994**, 48, 149.

14 Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, 50, 2394.

15 Berthelot, J.; Guette, C.; Desbène, P. L.; Basselier, J. J.; Chaquin, P.; Masure, D. *Can. J. Chem.* **1989**, 67, 2061.

Kaichang Li

Work Address

Department of Wood Science and Forest Products

Virginia Polytechnic Institute & State University

Blacksburg, VA 24061-0323

Phone: (540)231-3799

E-Mail Address: kli@vt.edu

EDUCATION

PhD, Wood Chemistry, Department of Wood Science and Forest Products, Virginia Polytechnic Institute & State University. Completion date: September 20, 1996.

MS, Organic Synthesis, Department of Chemistry, South China University of Technology, Guangzhou, P.R. China. June 1987

BS, Applied Chemistry, Department of Chemistry, South China University of Technology, Guangzhou, P.R. China. June 1984

PREVIOUS EXPERIENCE

Graduate Research Assistant. Department of Wood Science and Forest Products, Virginia Polytechnic institute & State University, Jan. 1993-present. Thesis work includes the synthesis of carbohydrate-lignin model compounds and neolignans. Syntheses require multi-step asymmetric transformations. Developed a stereospecific approach to β-*O*-4 lignin model dimers, as well as a practical method to synthesize 4-*O*-

methyl-α-D-glucopyranosiduronic acid. Synthesized ester-linked lignin-carbohydrate model compounds and studied their rearrangement reactions. synthesized neolignans in optical forms by use of carbohydrates as building blocks.

Research Associate. South China University of Technology/P.R. China. (1987-1992). Designed separation equipment to recover lignin from waste liquor in paper mills. Analyzed the composition and structure of lignin from waste liquor. Modified lignin structure through introducing new functional groups, changing molecular weight distribution and copolymerizing lignin with the other polymers. Several products such as Magnesium Lignosulfonate, Lignin Rust Preventer, Lignin Corrosion and Scale Inhibitor, and Lignin Cutting Fluid have been developed and marketed. Five research projects were completed.

Graduate Research Assistant South China University of Technology/P.R. China. (1984-1987). Synthesized novel phase transfer catalysts including crown ethers, quarternary ammonium salts and amines. Studied their catalytic behavior in nucleophilic substitution reaction. Solid state synthesis of phase transfer catalysts, *i.e.* grafted the synthesized catalysts to polystyrene resin so as to afford separation and reuse of the bound catalysts.

SKILLS

Laboratory. Familiar with operating NMR, HPLC, GC, FT-IR and DMTA equipment. In particular, capable of performing 1D and 2D NMR experiments on high field NMR spectrometers (^{1}H, ^{13}C, DEPT, TOCSY, COSY, HETCOR, HMQC, HMBC). Also experienced in interpreting NMR spectra. Extensive experience with low-pressure liquid chromatography (flash chromatography). Adept at organic synthesis including lignin and carbohydrate model compounds.

Computer Skills. Literate with Windows, DOS, Macintosh environments. Familiar with word processing software (MacWrite, Microsoft Word), drawing software (Chemintosh, MacDraw and Powerpoint), as well as spreadsheet and graphing packages (Excel and DeltaGraph).

HONORS

"Great Achievements in Scientific and Technological Progress" Award by State Education Commission of P.R. China. July 1989.

PUBLICATIONS

1. Li, K. and Helm, R. F. Use of Carbohydrates as Building Blocks to Synthesize Neolignans, in preparation.
2. Helm, R. F.; Toikka, M.; Li, K. and Brunow, G. Lignin Glycosides: Preparation and Optical Resolution. *J. Chem. Soc. Perkin Trans 1.* In press.
3. Li, K. and Helm, R. F. Approaches to Synthetic Neolignans. *J. Chem. Soc. Perkin Trans 1.* In press.
4. Li, K. and Helm, R. F. A Practical Synthesis of Methyl 4-*O*-Methyl-α-D-Glucopyranosiduronic Acid. *Carbohydr. Res.* 273(**1995**), 249-253.
5. Li, K. and Helm, R. F. Synthesis and Rearrangement Reactions of Ester-Linked Lignin-Carbohydrate Model Compounds. *J. Agric. Food Chem.* 48(**1995**), 2098-2103.
6. Helm, R. F. and Li, K. Complete *threo* Stereospecificity for the Preparation of β-*O*-4 Lignin Model Dimers. *Holzforschung.* 49(**1995**), 533-536.
7. Helm, R. F. and Li, K. Synthesis and Rearrangement Reactions of Lignin-uronic Acid Model Compounds Related to Hardwood Cell Wall Structure. *The 8th International Symposium on Wood and Pulping Chemistry.* Helsinki, Finland, June **1995**, vol. 1, pp107-114.

8. 10 papers published in Chinese Journals and books by 1993.

PRESENTATIONS/POSTERS

1. Li, K. and Helm, R. F. Use of Carbohydrates as Building Blocks to Synthesize Neolignans. 211th ACS National ACS Meeting, New Orleans, March 24-28, 1996. CELL-079.

2. Li, K. and Helm, R. F. Approaches to Synthetic Neolignans, 34th National Organic Symposium, Williamsburg, VA. June 11-15, 1995. Poster 281.

Kaichang Li